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Male Field Crickets Infested by Parasitoid Flies Express Phenotypes that May Benefit the Parasitoids

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Abstract

Parasites can cause changes in the phenotypes of their hosts that may benefit the parasite, the host, or both. To understand the evolutionary dynamics of host–parasite interactions it is necessary to first examine the effect of parasitic infestation on the host phenotype and whether the host or parasite benefits from these changes. The fly *Ormia ochracea* parasitizes the variable field cricket, *Gryllus lineaticeps*, and it uses male song to locate hosts for its lethal larvae. Adult flies preferentially orient to male songs with faster and longer chirps. We tested the effect of larval infestation on two types of host traits. First, we tested whether infestation affects male singing activity and song characters. Infested males were significantly less likely to sing than noninfested males, and when they did sing, they sang less frequently. Infestation thus reduced a male's ability to attract mates, which may benefit the parasitoid if mating activity increases predation, superparasitism and/or energetic costs for their hosts. No song character we measured, however, differed between infested and noninfested males. Second, we tested whether infestation affects host mass. Infested males gained more mass than noninfested males, which was not explained by the reduced singing of infested males. Importantly, parasitoids that developed in males that gained more mass were heavier as pupae, which may increase their viability and reproductive success as adults. These changes in the host may be beneficial side-effects of the pathology of parasitism, the result of a host-compensatory response, or the result of host manipulation by the parasitoid.

Keywords: Acoustic communication, Eavesdropping, Field cricket, *Gryllus lineaticeps*, *Ormia ochracea*, Parasitoid

Animals that are infected by parasites often differ from uninfected animals in their behavior, morphology and/or physiology (Thomas et al. 2005). Some of the most spectacular changes in host phenotype include the expression of submissive behavior (Libersat et al. 2009), host paralysis (Piek et al. 1971), induced suicide (Hohorst & Graefe 1961; Moore 1995; Biron et al. 2005), the building of safe pupation sites for parasitoids (Eberhard 2000, 2001), changes in host coloration to mimic a food item of a subsequent host (Yanoviak et al. 2008) and/or changes in morphology to attract predators (Wesenburg-Lund 1931; Kagan 1951). The phenotypic consequences of parasitism could be host manipulations caused by traits encoded in the parasite's genome (i.e. the 'extended phenotype' hypothesis; Dawkins 1982), or fortuitous by-products of infection that may result in benefits for the parasite (Poulin 2010). Recently, it has been suggested that both the parasite and the host may gain benefits if host changes mitigate the costs of infection for the host and concomitantly increase the parasite's transmission rate (Lefèvre et al. 2009), or if the parasite forces the host to collaborate (i.e. 'mafia-like' manipulation; Zahavi 1979; Thomas et al. 2005; Lefèvre et al. 2009). Changes of the host could also represent host adaptations for resisting or coping with parasites (e.g. Poulin et al. 1994; Wellnitz 2005; Poulin 2010). Finally, changes in the host phenotype may be the product of pathological side-effects of infection that are nonadaptive for either side (Minchella 1985). However, it has been argued that pathological side-effects that increase the reproductive success of the host and/or parasite will not be selected against (Combes 2001; Moore 2002; Klein 2005), and, if they have a genetic basis, may become adaptive (Poulin 2010).

Which side of the parasite-host interaction benefits from the changes in the host phenotype is often not clear and is the subject of an ongoing debate (e.g. Poulin 1995, 2010; Thomas et al. 2005; Lefèvre et al. 2009). The mechanisms mediating changes in the host are often highly complex, making it difficult to identify which side is responsible for the changes and who benefits from them (Lefèvre et al. 2009). Additionally, it is difficult to distinguish between some of the alternative explanations for host changes. For example, some cases of host changes have been interpreted as the result of manipulations sensu stricto (Dawkins 1982) or adaptive host responses, whereas these cases could also be interpreted as parasites exploiting the host compensatory response to parasitism (Lefèvre et al. 2009). Nevertheless, the first step to un-

derstand the dynamics of the parasite-host relationship is to determine whether the host phenotype changes as a result of parasitism, and whether these changes are beneficial for the parasite and/or the host.

The tachinid fly *Ormia ochracea* is a parasitoid that uses field crickets as hosts. Its larvae live and develop inside the host and kill the host when they emerge and pupate into free-living adults (Adamo et al. 1995b). *Ormia ochracea* ranges in North America from Florida to California and Hawaii and it parasitizes at least six species of field crickets across this range (Cade 1975; Walker 1986; Walker & Wineriter 1991; Zuk et al. 1993; Wagner 1996; Hedrick & Kortet 2006). In different geographical regions, the fly uses a different species as a host for its larvae. It locates its hosts using the mating songs of male crickets, and male parasitism rates can be as high as 80% in some species (Cade 1975). Once the fly lands near a male cricket, it expels two to three planidial larvae on the male and approximately six larvae on the ground around the male (Adamo et al. 1995a). Once the larvae make contact with a cricket, they burrow into the cricket's body and develop for the first 3 days within the thoracic flight muscles (the first phase of infestation) before they move to the abdomen (the second phase of infestation) to continue their development (Adamo et al. 1995b). Tissue damage due to larval feeding takes place only during the second phase of the infestation and primarily targets thoracic and abdominal muscles and fat tissue (Adamo et al. 1995b). The larvae emerge from the cricket approximately 7 days after infestation and kill the host during this process (Adamo et al. 1995b). After emergence, the larvae pupate and then eclose into adult flies.

We examined the effects of larval infestation on the behavior and morphology of male variable field crickets, *Gryllus lineaticeps*. This cricket species is a major host for California populations of *O. ochracea* (Wagner 1996; Wagner & Basolo 2007a; Martin & Wagner 2010). We specifically examined changes in host traits that should affect the fitness of the parasitoids. First, we tested whether infestation with *O. ochracea* larvae influences male singing activity and song characters. Changes in male song may be beneficial for the larvae in the context of superparasitism (i.e. infestation of a previously infested host; Fiske 1910). Larvae that parasitize a cricket within 24 h of the initial infestation incur 100% mortality (Adamo et al. 1995a), and the initial residents may experience increased competition, which could influence their size and, thus, fitness (see below). There is no evidence that the flies can distinguish between parasitized and nonparasitized crickets using nonacoustic cues (Adamo et al. 1995a). However, the flies usually prefer the same song types that female crickets prefer (e.g. Wagner 1996; Gray & Cade 1999; Wagner & Basolo 2007a, b), and larval infestation may cause changes in host singing activity or song characters that reduce the probability of a subsequent infestation by other flies. In addition, changes in singing activity or song characters may reduce host energy expenditure (Hoback & Wagner 1997) and the risk of attracting predators.

Second, we tested whether the fly larvae cause changes in host mass, and whether pupal mass is affected by changes in host mass. Since the larvae develop inside the host, host size may determine the amount of food available to the larvae and, thus, pupal size (Welch 2006). Pupal size has major effects on a fly's fitness: bigger pupae have greater survival and develop into bigger adults, which may have higher fecundity (e.g. King 1993; Adamo et al. 1995b; Allen & Hunt 2001; Kolluru & Zuk 2001). The parasitoids could affect host size through at least two mechanisms: parasitism could result in reduced energy expenditure (e.g. in a reduction in singing and other costly activities) or in increased foraging activity.

Methods

Study Animals

We collected adult female *O. ochracea* at Rancho Sierra Vista in the Santa Monica Mountain National Recreation Area (near Newberry Park, California, U.S.A.) in the summer of 2010, using broadcasts of *G. lineaticeps* song (Wagner & Basolo 2007b). The flies were brought to the University of Nebraska-Lincoln for experiments. The flies were kept in individual containers (13 × 17 × 8 cm) and fed with applesauce (Best Choice., Fort Worth, Texas, U.S.A.) and cotton (Padco., U.S. Cotton (Canada) Co., Lachine, Québec, Canada) soaked with a saturated sugar solution until the start of experiments. The fly food was replaced every 2 days.

We collected adult female *G. lineaticeps* from the same site as the flies in the summer 2008 to establish laboratory populations. Most of the female crickets mated before capture in the field and laid fertile eggs in the laboratory. Individuals hatching from those eggs constituted the first laboratory generation. We actively managed pairings between males and females for subsequent laboratory generations to reduce inbreeding. We used males of the second and older laboratory generations in our experiments. Crickets were reared to adulthood using the protocol described in Beckers & Wagner (2011). In brief, last-instar juvenile males were placed into individual containers and checked daily for adult moult. Individual containers had a paper towel substrate and cardboard shelters and the crickets were provided with water and cat chow (Nestlé, Purina Pet-Care Co., St. Louis, Missouri, U.S.A.) ad libitum. We kept all adult males until their death in environmental chambers set to a 14:10 h light:dark cycle at an ambient temperature of 21.1-27.2 °C and a relative humidity of 33-70%.

Infestations

We artificially infested crickets to examine the effects of the parasitoid larvae on cricket singing behavior and mass. Crickets were 7-12 days of adult age at the beginning of the experiments. We randomly assigned males to one of two treatment groups: infested ($N = 27$) and noninfested ($N = 26$). The age of the males did not differ significantly between treatment groups (infested: average ± SE: 9.07 ± 0.287 days; noninfested: 8.88 ± 0.325 days; Mann–Whitney U test: $U = 673$, $P = 0.605$). Males tested were drawn from 19 full-sibling families. We used no more than two males from the same family for either treatment (on average, infested: 1.4 males/family; noninfested: 1.3 males/family).

We killed each fly by removing its head and then dissected its abdomen to obtain planidial larvae for the infestation of the male crickets (for a detailed description see Vincent & Bertram 2009). On the day of infestation, we weighed the crickets and used a probe to transfer larvae to the crickets. Larvae were deposited on the dorsal surface of the cricket, along the membranous area between head and thorax (Vincent & Bertram 2009). We transferred three larvae to each cricket, which corresponds to a natural density of larvae found in cricket hosts infected by *O. ochracea* (1-3 larvae; Adamo et al. 1995a; Kolluru & Zuk 2001). Since larvae can move around on the cricket and may not successfully enter the host (Vincent & Bertram 2009), the number of larvae that emerged from some crickets was lower than the number of larvae transferred. However, larvae emerged from all infested crickets and all infested crickets died 7-10 days after initial infestation. Between two and three larvae emerged from most of the infested crickets. In two cases, four larvae emerged, which could be explained by errors in the number of larvae transferred. We included these individuals in our analyses, which did not change our results. Crickets from the noninfested treatment were handled in the same way as the in-

fested crickets except that there were no larvae on the probe. Data from males that lost body parts (e.g. a leg) or that died during the experiment were excluded from analysis (infested: 3 males; noninfested: 1 male). 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.

Recording of Male Song and Singing Activity

To examine the effect of parasitoid larvae on male singing behavior, male songs were recorded, and male singing activity was measured 1, 3 and 5 days after infestation or sham treatment. On each day of recording, we placed the container holding a male in 1 of 10 Styrofoam rectangular coolers (50 × 33 × 40 cm) that had been lined with acoustic foam. The acoustic foam prevented males from hearing singing males in adjacent chambers. We replaced the plastic lid of the individual container with a wire mesh lid to reduce reverberations. A microphone (Sennheiser ME64 K6P or Schriber acoustic SA-568) was suspended above each cricket through a hole in the lid of the Styrofoam box. Each male was recorded in the dark during the dark portion of the light:dark cycle. The microphones were connected through a 10-channel recording board (Micro 1401 and expansion ADL 12, both Cambridge Electronic Design Ltd, Cambridge, U.K.) to a personal computer (Macintosh G3). Songs were digitized and analyzed using Spike 2.0 (Cambridge Electronic Design Ltd, 1995). The following song characters were measured: chirp rate, chirp duration, pulse duration, interchirp interval and dominant frequency. We only analyzed recordings that were at least 20 s in duration. All recordings took place between 1200 and 1930 hours. Ambient temperature was measured at the beginning of each recording period (range 21.9-23.0 °C). On each of the 3 days, we recorded each male for four 50 min periods (200 min total of recordings each day). During recordings, we broadcast a synthesized chorus of five males in the recording room to stimulate experimental males to sing. The songs within the chorus had different temporal patterns and overlapped with each other in a pseudorandom fashion (i.e. no single song stood out from the chorus). The chorus sound was broadcast at peak amplitudes of 80 ± 2 dB (re: 20 mPa) measured at 30.5 cm from the speaker using a CEL-254 sound level meter.

To estimate the probability of singing for each male, we scored the presence or absence of at least one chirp during the 200 min of recordings on a given day (1 = yes, 0 = no). To estimate the amount of singing for each male, we counted the number of recording periods during which the male produced at least one chirp (range 0-4). For example, if a male produced song during two of the four recoding periods on a given day, his singing activity was scored as 2.

Measurements of Cricket and Fly Pupae Mass

All males were weighed to the nearest 0.0001 g on the day of infestation using an electronic balance (Sartorius BP-61). Fly larvae weighed less than 0.0001 g, which was less than the smallest value the electronic balance could measure. Initial male mass did not differ significantly between treatment groups (infested: $N = 27$, 0.489 ± 0.0159 g; noninfested: N = 26, 0.519 ± 0.0224 g; Mann–Whitney U test: $U = 750$, $P = 0.398$). We weighed males of both treatment groups again 5 days after infestation. We checked crickets every day between 1200 and 1800 hours for emerged larvae. No larva emerged during the 5 days of song recordings.

Fly larvae pupate within 1 h after emergence, and host crickets die within a few hours of emergence (O. M. Beckers, personal observation). We counted the number of pupae and measured their mass on the day of emergence. For each

cricket, we averaged the mass of all pupae. We checked for additional larvae over the 2 days following emergence of the first set of larvae. Our sample size varied among analyses because we missed one male cricket mass measurement and one pupal mass measurement (both from infested males).

Statistical Procedures

We used linear mixed models with maximum likelihood estimation to examine the effects of parasitoid larvae on male cricket song characters and singing activity. These models had five fixed effects: treatment (infested or noninfested), recording day (day 1, 3 or 5), male age, average temperature for each recording day, and the interaction between treatment and day. The models also included male family as a random factor to account for using up to two males from the same family. We also included male identity as a random factor to account for the repeated measurement of individual males (i.e. the measurement of male singing activity and song characters on each of 3 days). The probability of singing was analyzed using a generalized linear model with binomial errors. The amount of singing, which was a count variable, was analyzed using a generalized linear model with Poisson errors. Male song characters, which were continuous and normally distributed variables, were analyzed using a generalized linear model with Gaussian errors. We also examined the effect of pupa number on the probability of singing and singing activity, using only males in the infested treatment. In these models there were two random effects (male identity and family) and five effects (number of pupae that emerged, recording day, male age at infestation/sham infestation, ambient temperature of recording day, and the interaction between the number of pupae and day).

To examine the effect of infestation on male mass gain (day 5 mass – day 1 mass), we used a model with treatment, initial cricket mass on day of infestation and male age at infestation as fixed effects, and family as a random effect.

We also examined the effects of larvae number, initial male mass, male mass gain and male age at infestation (fixed effects) on the average pupal mass. We included male family as a random factor to this model.

Finally, we used another linear mixed model to test for the effect of singing activity on male mass gain. Within each treatment, we compared the mass gain of males that sang on at least 1 day to that of males that never sang. We included male family as a random factor in the model.

We removed stepwise nonsignificant interactions from our models. Only reduced models are presented. All statistical analyses were performed using the software packages Stata v.10 (StataCorp LP, College Station, Texas, U.S.A.) and JMP v.8.0.2 (SAS Institute, Cary, North Carolina, U.S.A.). All statistics are presented as means ± SE.

Results

Larval Infestation and Male Singing Behavior

We compared the singing activities and song characters of parasitized and nonparasitized males on each of the three recording days (1, 3 and 5 days after infestation or sham infestation). The probability of singing significantly differed between the treatment groups (Table 1). Infested males were less likely to sing than noninfested males during each of the three recording days (Figure 1a). In addition, there was no significant effect of the interaction between treatment and day, indicating that the effect of parasitism on the probability of singing varied little over the 5-day period. Older males were more likely to sing than younger males, but male age did not differ between treatment groups (see Methods). Ambient temperature had no significant effect on the probability of singing (Table 1). In a sep-
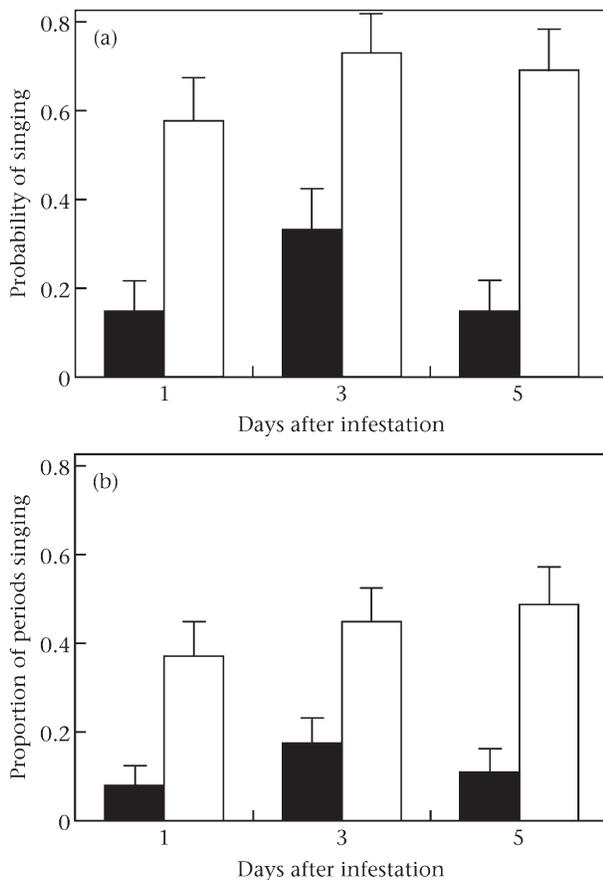
Table 1. Results of a linear mixed model examining effects on the probability of singing in *Gryllus lineaticeps*

Fixed effects	Coefficient	SE	χ^2_1	P
Treatment	−2.711	1.264	4.60	0.032
Recording day	0.536	0.475	1.27	0.259
Age at infection	0.604	0.261	5.37	0.021
Temperature	0.830	1.392	0.36	0.551
Day*treatment	−0.276	0.301	0.84	0.359
Random effect	**Estimate**	**SE**		
Male	1.315	0.659		
Family	1.636	0.606		

Regression coefficients are shown for the fixed effects; the variance estimate is shown for the random effect.

Table 2. Results of a linear mixed model examining effects on male singing activity in *Gryllus lineaticeps*

Fixed effects	Coefficient	SE	χ^2_1	P
Treatment	−1.610	0.505	10.18	0.001
Recording day	0.069	0.146	0.22	0.636
Age at infection	0.270	0.100	7.22	0.007
Temperature	−0.090	0.428	0.04	0.834
Day*treatment	−0.006	0.112	0.00	0.960
Random effect	**Estimate**	**SE**		
Male	0.654	0.217		
Family	0.748	0.252		

Regression coefficients are shown for the fixed effects; the variance estimate is shown for the random effect.

arate analysis using males in the parasitized group, there was no effect of the number of pupae, day, temperature, or the interaction between the number of pupae and day on the probability of singing (all $\chi^2_1 \leq 1.41$, all $P \leq 0.235$). There was, however, a positive effect of age on the probability of singing ($\chi^2_1 = 9.50$, $P = 0.002$).

As with the probability of singing, singing activity significantly differed between the treatment groups (Table 2). Infested males sang significantly less frequently than noninfested males during each of the three recording days (Figure 1b).

Figure 1. Effect of infestation with *O. ochracea* larvae on male singing activity in *G. lineaticeps*. (a) Proportion of infested and noninfested males that produced song on each of three recording days following infestation. (b) Proportion of time intervals during which infested and noninfested males sang on each of three recording days following infestation. ■: infested males; □: noninfested males. Values are means ± SE.

In addition, there was no significant effect of the interaction between treatment and day, indicating that the effect of parasitism on male singing activity varied little over the 5-day period. Ambient temperature had no significant effect on the amount of singing, but older males sang more frequently than younger males. In a separate analysis using males in the parasitized group, there was no effect of the number of pupae, day, temperature, or the interaction between the number of pupae and day on the probability of singing (all $\chi^2_1 \leq 2.92$, $P \geq 0.088$). There was, however, again a positive effect of age on singing activity ($\chi^2_1 = 5.53$, $P = 0.019$).

There were no significant effects of treatment, recording temperature and/or male age on any of the song characters measured (chirp rate, chirp duration, chirp interval, pulse duration, and dominant frequency: all $\chi^2_1 \leq 1.22$, all $P \geq 0.270$). Thus, while parasitism affected whether males sang and how frequently they sang, it did not affect the types of songs that males produced when they did sing.

Larval Infestation and Male Mass
First, we examined whether initial mass, number of larvae, age at infestation or mass gain of the male affected pupal mass. Initial mass of the male had a significant positive effect on pupal mass ($\chi^2_1 = 22.96$, $N = 26$, $P < 0.0001$; Figure 2a), whereas number of larvae ($\chi^2_1 = 41.86$, $P < 0.0001$; Figure 2a) and male age at infestation ($\chi^2_1 = 13.02$, $P = 0.0003$) had a significant negative effect on pupal mass. Most importantly, there was a significant positive effect of male mass gain on pupal mass ($\chi^2_1 = 25.66$, $P < 0.0001$); the more mass the male gained after infestation, the heavier the pupae were on the day of emergence (Figure 2b).

The positive effects of initial mass and mass gain of males on pupal mass suggest that larvae benefit from being in larger hosts that gain more mass following infestation. While the larvae cannot affect the host's initial mass, it is possible that they might affect how a host's mass changes following infestation. We tested this hypothesis using a linear mixed model that examined the effects of treatments, male mass and male age at infestation on male mass gain. There was a significant effect of treatment on male mass gain: infested males gained significantly more mass than noninfested males ($\chi^2_1 = 34.99$, $N_{infested} = 27$, $N_{noninfested} = 26$; $P < 0.0001$; Figure 3). However, there were no significant effects of initial male mass ($\chi^2_1 = 0.09$, $P = 0.769$), or male age at infestation ($\chi^2_1 = 0.00$, $P = 0.985$) on male mass gain.

Male Singing Activity and Mass Gain
Because infested males were less likely to sing, an energetically expensive activity (e.g. Prestwich 1994; Hoback & Wagner 1997), we tested whether infested males gained more mass because they sang less frequently. There was no signifi-
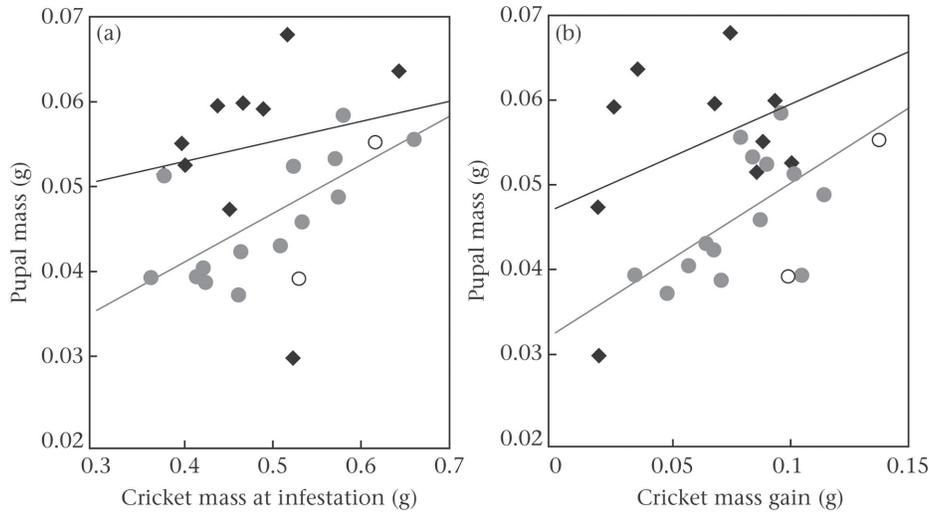
Figure 2. Effect of host mass on the mass of *O. ochracea* pupae. (a) Relationship between host mass on the day of infestation and average pupal mass. (b) Relationship between host mass gain and average pupal mass. Note that regression lines re.ect the general pattern in each graph. We did not include regression lines for the two males that produced four pupae. ♦: two pupae; ●: three pupae; ○: four pupae. Values are means ± SE.

cant difference in mass gain ($\chi_1^2 = 0.94$, $P = 0.331$), initial cricket mass ($\chi_1^2 = 0.20$, $P = 0.655$) or age at infestation ($\chi_1^2 = 0.28$, $P = 0.595$) between singing and silent males in the infested treatment group ($N = 27$). There was a marginally significant difference in mass gain between singing and silent males in the noninfested group ($\chi_1^2 = 3.93$, $N = 26$, $P = 0.047$). However, this result was in the opposite direction to that hypothesized: silent males gained less mass (mean difference = −0.0274 ± 0.032 g, $N = 6$) than singing males (0.0199 ± 0.006 g, $N = 20$). Neither initial cricket mass ($\chi_1^2 = 0.11$, $P = 0.745$), nor age at infestation ($\chi_1^2 = 0.16$, $P = 0.693$) significantly affected male mass gain in noninfested crickets. There was thus no evidence that infested males gained more mass because they sang less frequently.
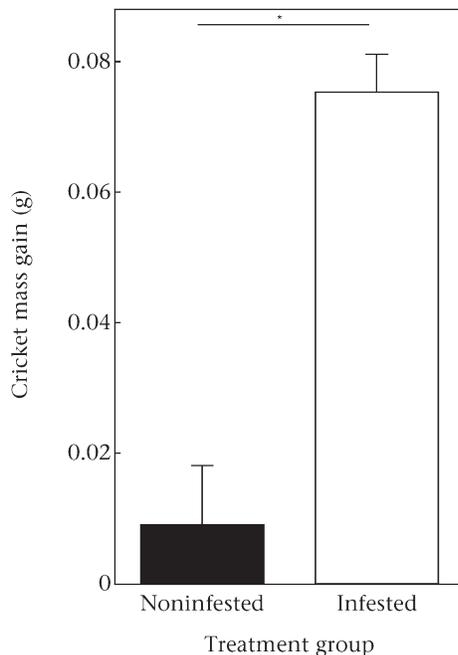
Figure 3. Mean ± SE mass gain of noninfested and infested males. Asterisk indicates a significant difference ($P < 0.05$) between the groups.

Discussion

Our experiments demonstrated that larval infestation caused changes in the phenotype of *G. lineaticeps*. Parasitized males were less likely to sing, and sang less frequently, than nonparasitized males. These effects of parasitism on male singing activity were present on the day following infestation and persisted during all subsequent days in which singing activity was measured. If male singing activity affects the probability of superparasitism or the probability that a predator eats the host, the parasitoids may benefit from this effect. There was, however, no detectable effect of parasitism on any of the song characters measured.

In addition to the effects of parasitism on male singing activity, parasitized males gained more mass than nonparasitized males. Because there was a positive effect of host mass gain on parasitoid size at pupation and because pupal size can affect adult fitness (e.g. Allen & Hunt 2001; Kolluru & Zuk 2001), the parasitoids may benefit from this effect. It remains to be determined, however, whether these changes in host phenotype are beneficial side-effects of the pathology of parasitism, a result of exploiting a host-compensatory response, or whether they are a consequence of parasitoid adaptations for manipulating the phenotypes of their hosts.

Larval Infestation and Host Singing Behavior

In other field cricket species that are used by *O. ochracea* as hosts, infestation also results in a reduction of male singing activity, but there is variation in the timing of this effect. In parasitized *Gryllus texensis* crickets, singing is either gradually reduced during the course of infestation (Cade 1984), or substantially reduced during the second phase of infestation (i.e. when the larvae move to the abdomen and begin eating muscle tissue; Orozco & Bertram 2004). In *Teleogryllus oceanicus*, male singing activity was initially observed to be comparable between parasitized and nonparasitized males (Kolluru 1999), whereas later studies showed that male singing dropped substantially soon after infestation and remained low (Zuk et al. 1995; Kolluru et al. 2002), similar to our results for *G. lineaticeps*. There thus appears to be variation among field crickets and/or among populations of flies in the effects of parasitism. It is not known how long *O. ochracea* has been interacting with each of the host species, but these differences could potentially be explained by

differences in the time that each has had to adapt to the other (sensu Adamo 1999; Kolluru et al. 2002). Additionally, species of field crickets, and populations of flies, could vary in a number of traits that affect the singing activity of infected males (e.g. species of field crickets may vary in their immune responses or general physiology, while populations of flies may vary in the time at which they begin damaging their hosts).

There are a several non-mutually exclusive explanations for the effect of infestation on male singing activity in *G. lineaticeps*. First, the reduction in singing activity could be a pathological side-effect of infestation. Larvae of *O. ochracea* reside in the thoracic wing muscles during the first phase (the first 3 days) of the infestation (Adamo et al. 1995b). Even though the muscle tissue is not visibly destroyed (Adamo et al. 1995b), inflammation or functional damage of the tissue may cause infested crickets to move these muscles less, resulting in a decline of singing activity. However, song characters that require more muscle movement (such as the production of more chirps per unit time and longer chirps) were not affected by infestation, which may be inconsistent with the damage hypothesis. In addition, infested *Gryllus rubens* are capable of using their thoracic muscles for flight for approximately 3-5 days after infestation (Walker & Wineriter 1991), which may also be inconsistent with the damage hypothesis. It also conceivable that infestation slows down the host metabolism (Kolluru et al. 2004) and thus may reduce the capacity of the host to break down storage tissue in order to free energy (Kolluru et al. 2002) for costly activities such as singing (e.g. Prestwich & Walker 1981; Forrest et al. 1991; Hoback & Wagner 1997; Hack 1998).

Second, singing in infested male crickets may be reduced because of an adaptive host response to mitigate the detrimental effects of the infestation (Lefèvre et al. 2009). For example, infested hosts may allocate more of the available energy to boost their immune response, leaving less energy available for singing.

Third, *O. ochracea* larvae may interfere with the host's nervous system (physically or through neurotransmitters), immune system, endocrine system, or metabolism (Moore 2002; Beckage & Gelman 2004; Libersat et al. 2009) to induce changes in host singing behavior (i.e. manipulate the host). Singing in crickets is energetically expensive (e.g. Prestwich & Walker 1981; Hoback & Wagner 1997) and shortens the life span of males (Hunt et al. 2004). Additionally, silent males do not attract females for mating, and silent males in *G. lineaticeps* do not appear to display alternative reproductive tactics (Wagner 2011). As a result, parasitized males should engage in fewer expensive reproductive activities, resulting in more energy that could be used by the parasitoids (Thomas et al. 2005).

In addition, conspicuous mating songs can attract eavesdropping predators and other *O. ochracea* females that may super-parasitize infested male crickets. Previous studies of tachinid flies have shown that the number of infesting larvae is negatively correlated with pupal size, and that smaller pupae have higher mortality rates and lower adult fecundities (e.g. Adamo et al. 1995a; Allen 1995; Kolluru & Zuk 2001; Welch 2006; Lehmann 2008). Superparasitism (i.e. multiple infestations) by *O. ochracea* also increases the number of larvae inside the host, and resident larvae may benefit from preventing additional infestations. However, more experiments are necessary to test the effects of superparasitism on larval competition and to identify the mechanism that causes reduced singing of infested males.

Data on the effect of infestation on song characters is very limited. To our knowledge, the only study that tested for song character effects found that infested male katydids, *Poecilimon marianne*, produced songs that were less attractive to females when infested by a tachinid fly, *Theorobia leonidei* (Lehmann &

Lehmann 2006). However, since the songs of the males were not recorded and analyzed, it is not known which song characters may have changed as result of infestation. In contrast, our results show that songs of infested *G. lineaticeps* males did not differ in either spectral or temporal aspects from the songs of noninfested males. Chirp rate in *G. lineaticeps* songs shows plasticity in response to variation in the nutritional environment (Tolle & Wagner 2011). This finding suggests that a lack of change in male song characters in response to parasitism is not a result of an inability of male song characters to change in response to variation in intrinsic factors. However, the same study on *G. lineaticeps* (Tolle & Wagner 2011) showed that different genotypes responded to nutritional variation to a different degree. It is possible that some genotypes respond to parasitism by increasing their chirp rates, while other genotypes respond to parasitism by decreasing their chirp rates, resulting in no detectable net population effect. Nevertheless, our findings suggest that even though infested males reduce their singing activity, the average attractiveness of the songs they produce is unaffected by infestation.

Larval Infestation and Host Mass
We found that infested males gained more mass than noninfested males and that pupal mass was positively affected by the amount of mass gained by the host. Both the hatching success of adult flies from pupae and adult size are positively correlated with pupal size (e.g. Adamo et al. 1995b; Allen & Hunt 2001; Kolluru & Zuk 2001; Lehmann 2008). In tachinid and hymenopteran parasitoids, larger females tend to be more fecund (tachinids: e.g. King et al. 1976; Allen 1995; Nakamura 1995; Allen & Hunt 2001; Kolluru & Zuk 2001; hymenopterans: reviewed in Godfray 1994) and more active in host searching (Allen & Hunt 2001), and larger males may be more successful in controlling better quality territories than are smaller males (Allen & Hunt 2001). If size affects fitness in *O. ochracea*, as it affects the fitness of many other parasitoids, then the gain in host mass may benefit the parasitoids.

The increased mass gain in infested crickets could potentially be explained by the reduced singing activity of parasitized males. Because singing is energetically expensive (e.g. Hoback & Wagner 1997), parasitized males that sing less frequently will use less stored energy. However, there was no effect of singing activity on male mass gain in either infested males or noninfested males, suggesting that reduced singing may contribute little to the mass gain of infested crickets. Other potential explanations are that infested males reduce their metabolic rate in response to infestation (Kolluru et al. 2004), increase their food intake and/or reduce their general activity, and thus, the energy they use (but see Martin & Wagner 2010). Additional work is necessary to determine whether the mass gain of infested males is a host response against the parasitoid, a parasitoid-induced response in the host, or both.

Who Benefits from Changes in Host Phenotype?
To understand the coevolution of the host and its parasite, it is necessary to establish how the species affect each other's phenotypes in this interaction. We found that infestation with *O. ochracea* larvae substantially reduced male singing activity and increased host mass gain in *G. lineaticeps*. As discussed above, these changes in host phenotype may result in multiple benefits for the parasitoids. Whether the host benefits from these changes is not clear. All infested *G. lineaticeps* males died as a result of infestation. In addition, infested males showed minimal singing activity 24 h after infestation, which should result in a low probability of attracting females following infestation. Given the effect of infestation on survivorship, and the inferred effect of infestation on reproductive success, it seems

unlikely that the changes we observed in infested males are adaptive in the context of infestation by *O. ochracea*. It is possible, however, that these changes are part of a general immune response that is adaptive in the context of other parasites or pathogens. It is thus premature to conclude that the parasitoids manipulate the traits of their hosts, although our results provide preliminary support for this hypothesis.

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