Reduced reproductive effort in male field crickets infested with parasitoid fly larvae

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Some populations of the field cricket *Teleogryllus oceanicus* are parasitized by the phonotactic fly *Ormia ochracea*. Flies locate crickets by their song and deposit larvae onto them. The larvae develop inside the cricket for 1 week before killing the host upon emergence. The reproductive compensation hypothesis predicts that parasitized crickets should increase their reproductive effort during the initial stages of infestation to offset the loss of fitness resulting from their shortened life span. An alternative hypothesis predicts that parasitized crickets will decrease reproduction, either because they are unable to reproduce or because selection acting on the parasitoid favors decreased host reproduction. In laboratory experiments, parasitized male crickets had reduced reproductive effort (spermatophore production, calling, mating activity, and mass allocated to reproductive tissue) compared to unparasitized males. Parasitized males fed ad libitum showed no evidence of allocating a greater proportion of their resources to reproduction. Parasitized and healthy males did not differ significantly in resting or maximal metabolic rates, although this may have been due to the substantial contribution of larval respiration to the metabolic rate of the host–parasitoid complex. These results are consistent with previous studies and suggest that *T. oceanicus* males parasitized by *O. ochracea* do not increase their reproductive effort. We discuss potential reasons that crickets do not increase reproductive effort in response to fly larvae and address difficulties in demonstrating altered life-history patterns in response to parasitism. *Key words:* crickets, mating, phonotactic parasitoids, reproductive compensation, reproductive effort, *Teleogryllus*.

Parasitism can significantly influence host life-history patterns (Agnew et al., 2000; Forbes, 1993; Minchella, 1985). Traditionally, changes in patterns of reproduction in parasitized animals were viewed as either byproducts of damage or as parasite adaptations to reduce predation and to free host resources for parasite growth (Fritz, 1982; Horton and Moore, 1993; Minchella, 1985). Indeed, some parasites are castrators, completely destroying the host's reproductive machinery (Kuris, 1974). However, more recent studies have explored whether changes in host reproduction represent host, rather than parasite, adaptations (Forbes, 1993; Poulin, 1995).

Minchella and Loverde (1981) hypothesized that parasitized animals should be selected to increase current reproductive effort to offset future losses and demonstrated that snails exposed to trematode parasites increased egg laying. Their "reproductive compensation hypothesis" rests on the concept of residual reproductive value (Fisher, 1958; Williams, 1966). Because the residual reproductive value of a parasitized individual is decreased, it would be adaptive for that individual to shift more of its resources into the current reproductive event. This hypothesis has since been supported in several studies (reviewed by Agnew et al., 2000). For example, crickets infected with bacteria, *Drosophila* infested with mites, and amphipods harboring trematodes all increase reproductive activity (Adamo, 1999; McCurdy et al., 2000; Polak and Starmer, 1998).

Teleogryllus oceanicus is an Australian field cricket that has been introduced into Hawaii, where it is parasitized by the New World tachinid fly *Ormia ochracea,* also introduced into Hawaii (Otte and Alexander, 1983; Zuk et al., 1993). Gravid female *O. ochracea* acoustically orient to *T. oceanicus* calling song and deposit larvae on the cricket (Cade, 1975). The larvae develop inside the host for 1 week and kill the host upon emergence (Adamo et al., 1995). Ormiine flies significantly shorten host life span (Lehmann and Heller, 1997), and there is a sharp decrease in the frequency of older male crickets in populations parasitized by *O. ochracea* compared with unparasitized populations (Murray and Cade, 1995; Simmons and Zuk, 1994).

Parasitized crickets mount an encapsulation response in which *O. ochracea* larvae are enclosed by layers of hemocytes (Vinson, 1990). Contrary to the reproductive compensation hypothesis, parasitized female *Gryllus* crickets produced fewer eggs than control females (Adamo, 1999; Adamo et al., 1995). In the most detailed study to date of the effects of *O. ochracea* on male cricket reproduction, Adamo et al. (1995) showed that latency to courtship singing was unaltered in *Gryllus* species. However, these authors did not examine other, potentially more direct measures of reproductive effort. Other studies have demonstrated that infestation with late-stage ormiine parasitoid larvae depresses male orthopteran calling activity (Cade, 1984; Zuk et al., 1995) and spermatophylax weight (Lehmann and Lehmann, 2000).

We tested the reproductive compensation hypothesis against the alternative hypothesis that parasitization depresses host resource allocation to reproduction (either as a byproduct of infestation or as an adaptation on the part of the parasite; Adamo et al., 1995; Agnew et al., 2000; Cade and Wyatt, 1984). We examined the reproductive effort of parasitized and unparasitized *T. oceanicus* males by experimentally infesting groups of males and comparing their calling activity, spermatophore production, sperm viability, and mating activity to that of unparasitized males. Changes in host reproductive effort could be masked by parasitoid-induced changes in reproductive activity or physiology (Adamo, 1999; Forbes, 1996; Perrin et al., 1996). Accordingly, we also examined the effects of parasitoids on the host's capacity for metabolic power output (which may impact reproductive activities such as singing or courtship) and resting rates of energy expenditure and on allocation of resources to storage versus reproductive tissues.

METHODS

Experimental infestation

We obtained parasitoid flies used in the calling activity and fecundity experiments from a laboratory colony maintained by R. R. Hoy at Cornell University. We trapped flies used in all other experiments on the grounds of the University of Hawaii, Hilo, using ceramic tiles coated with Tanglefoot insect trap coating and baited with tape-recorded, synthesized *T. oceanicus* song. Unless otherwise stated, we collected crickets to be parasitized by hand from the same location as the flies and anesthetized them with CO_2 (100% for approximately 15 s) immediately before infestation. Larvae were dissected from the flies, and a dissecting pin was used to transfer three to five mobile larvae onto the membranous area around the front legs of each cricket (Cade, 1984). This number of larvae is within the natural range found parasitizing the wild Hilo population of *T. oceanicus* (Zuk et al., 1995). Unparasitized males were anesthetized and handled under a dissecting microscope.

At least one parasitoid larva emerged from each parasitized male, and unparasitized males did not harbor any parasitoid larvae. All infested crickets died within hours of larval emergence. The weights of pupae resulting from experimental infestations were heavier than those resulting from natural infestations, probably due to greater food intake by the laboratory-reared crickets (*t* test; $n = 19$ pupae from natural infestation, 20 from experimental infestation; $t = 5.23$; $p < .0001$).

Calling activity

We monitored calling activity using an electronic circuit connected to a Macintosh IIsi computer (after Kidder and Sakaluk, 1989). The device sampled each of eight microphones, sensitive to the frequency of *T. oceanicus* calling song (4–5 kHz), once per second, so that we were able to determine whether or not each male was calling in 1-s increments throughout the night. The crickets and the monitoring device were housed at the University of California, Riverside, in an anechoic chamber maintained at 31 ± 3°C and 12:12 h light : dark schedule.

Pilot tests using tape-recorded cricket song allowed for the adjustment of microphone sensitivity. To ensure that the microphones accurately recorded calling activity, we conducted a test of the monitoring device using four laboratory-reared *T. oceanicus* males monitored for 6 nights (12 h per night; after Bertram and Johnson, 1998). The eight microphones were grouped into pairs, and each pair was threaded through a rubber stopper with a hole in it and hung 3 cm from the top of each of four 0.5-l plastic containers with air holes. Each container housed one male cricket, a cardboard shelter, and ad libitum food and water. We grouped data from each container's two microphones into half-hour segments and compared them using correlation analysis. All of the 23 resulting correlations were significant after applying a Bonferroni correction for multiple correlation tests ($r_{\text{adjusted}} = 0.88 \pm 0.05$; $p = 2.63 \times 10^{-8} \pm 1.87 \times 10^{-8}$).

Calling activity experiments were conducted from February to May 1999. We obtained virgin male crickets from our laboratory colony as last-instar nymphs and housed them in a group container until they eclosed. The males were 1–7 weeks old at the start of the experiment, and males in the parasitized and control groups did not differ in age. Each male was individually isolated in a 0.5-l plastic container with food, water, and a cardboard shelter. One microphone was placed in each container as described above, and calling activity was monitored for seven continuous nights (approximately 12 h per night). On the eighth day of the experiment, the males were anesthetized using CO_2, handled under a dissecting microscope, and measured to the nearest 0.1 mm with digital calipers. Anesthesia and handling were performed to examine their effects on subsequent calling activity. Males were allowed to recover, and calling activity was monitored again for 2 nights. Males were then experimentally infested with parasitoid larvae as described above, and calling activity was monitored until the larvae emerged.

We repeated the experiment with a control group of males to examine the effects of repeated anesthesia and handling on calling activity. All experimental conditions including the ages of the males were similar to those in the parasitized male tests, except that these males were anesthetized and handled twice without being infested with parasitoid larvae. At the end of each experiment the males were individually housed until pupae emerged from all parasitized males. All males were then frozen, their pronotum width was measured using dial calipers, and they were dissected under a dissecting microscope to detect additional larvae. We compared the mean calling activity of males before and after parasitization (before and after second anesthetization for the control males) using Wilcoxon paired-sample tests.

Spermatophore production

We conducted spermatophore production experiments in Hilo, Hawaii, during July and August 1997. We collected crickets as adults from the same location as the flies a few days before each experiment. Although cricket ages were unknown, all males were calling at the time of collection. The crickets were maintained under the natural light : dark schedule (approximately 13:11 h) and kept on an ad libitum diet of dry cat food and water. *T. oceanicus* produce sperm in discrete spermatophores, which are small and simple, lacking the nutrient-rich spermatophylax portion found in other orthopterans such as katydids (Loher and Dambach, 1989). Because male crickets exhibit diel periodicity in spermatophore production and do not usually transfer spermatophores during daylight hours (Loher, 1989; McFarlane, 1968), we collected spermatophores from shortly before sunset (1700 h) until shortly after first light (0500 h). Collection involved visual examination of the male and female for protruding spermatophores, examination of the container floor for any dislodged spermatophores, and gentle squeezing of the abdomen of each male to extrude spermatophores that had not yet protruded (Cade and Wyatt, 1984; Zuk, 1987). We assumed that spermatophores from parasitized and unparasitized males were equally likely to be eaten.

We examined the effects of contact with females on spermatophore production in two separate experiments, each with a different set of crickets. In each case, we infested males between 1300 and 1500 h, and that evening was designated day 1 post-infestation. We collected spermatophores on days 4 and 6 post-infestation in the first experiment ($n = 15$ unparasitized, 12 parasitized males), and on days 1, 2, 3, and 5 post-infestation in the second experiment ($n = 14$ parasitized, 14 unparasitized males). Males in the first experiment were individually isolated for the few days between capture and the experiment, and males in the second experiment were housed in mixed-sex groups during those few days.

In both experiments, each male was placed in a 0.9-l Styrofoam container with one unparasitized female within 1 h of infestation. On the indicated nights post-infestation, we checked each pair once every hour and removed any spermatophores detected with forceps. Females were rotated regularly in both experiments to minimize female effects on mating activity, so that in the first experiment each male was with

three different females, and in the second experiment each male was with five different females.

Spermatophore data were normally distributed (analyses of residuals by Shapiro-Wilk test; experiment 1: $W = 0.9453$; $p = .1805$; experiment 2: $W = 0.9473$, $p = .2431$) and were analyzed using repeated-measures ANOVA (SAS Institute, 1990).

Mating activity

We examined mating activity in the laboratory in Hilo, Hawaii, in June 1998. We collected crickets as adults from the same location as the flies a few days before the start of the experiment and maintained them at 25 ± 1°C, 60% humidity and 12:12 h light : dark schedule throughout the experiment. All males were calling at the time of capture and were therefore assumed to be reproductively active. Each male ($n = 19$ parasitized, 16 unparasitized males) was individually housed in a 0.6-l plastic container with water, cat food, and a paper shelter. Just before the start of the first day of observations one mature female was introduced into each male's container. We rotated females regularly before the start of each day's observations so that each male was with four different females by the end of the experiment. We conducted behavioral observations every day between 2100 and 0030 h for 6 days. The parasite status of crickets was not known to the observer. Each pair of crickets was examined and behavior recorded every 10 min during the observation period. If the male was courtship singing during an observation period, then we monitored that couple either until mounting took place or until the next scheduled observation occurred. Mounting was scored if the female mounted the male and remained on him for more than 10 s. Failure was scored if the male performed courtship singing for two or more observations (≥ 20 min) without being mounted. Males in this category were usually not mounted for the remainder of the night.

Mating activity data were not normally distributed (mounting: $W = 0.92$, $p = .01$; failure: $W = 0.87$, $p = .0005$), and there was no significant difference across nights (Kruskal-Wallis test; mountings: $\chi^2 = 4.50$, $p = .48$; failures: $\chi^2 = 8.57$, $p = .20$). Therefore, we performed nonparametric tests on the totals for each male across the 6 nights of the experiment.

Fertilization success

We examined the effects of parasitoid infestation on male fertilization success by comparing the fertility and fecundity of females mated to parasitized (days 2–4 post-infestation) and unparasitized males. The experiment was conducted in an environmental chamber (27 ± 1°C, 70% humidity, and 12:12 h light : dark schedule) from February to May 1999. We obtained crickets from our laboratory colony as last-instar nymphs and housed them in single-sex group containers until they eclosed. Males were either parasitized or handled at 4–13 days of age, after which each male was housed with two virgin, unparasitized females (with the exception of one unparasitized male who was housed with only one female) for 2 nights in a 0.9-L plastic container with cat food, water, and shelter. Females were in either the young (4–13 days old) or old (33–60 days old) age class and ranged in size (pronotum width) from 5.11 to 5.99 mm. To minimize female age and size effects on fecundity, we distributed females in a stratified, random fashion between parasitized and unparasitized males, such that each group of males had an approximately equal number of females of each age and size class. After 2 nights the females were removed into individual 0.9-l plastic containers with food, water, shelter, and an egg-laying dish.

T. oceanicus females exhibit an egg flood, an increase in oviposition immediately after mating (Vaughan, 1995). Therefore, we replaced egg-laying dishes after 2 days, and each female had two dishes, representing the first 2 days of laying and the subsequent 13 days of laying. Because *T. oceanicus* females lay unfertilized eggs (Vaughan, 1995), we used hatchlings and not eggs to assess male fertilization success. However, because some clutches had no hatchlings, all unhatched eggs were also counted as an indication of female fecundity. Although pigmented eyespots can usually be used to determine if eggs contain embryos, the eggs from this experiment were often dark due to age, and eyespots were therefore not used as indicators of fertilization. At the end of the experiment we dissected all females to determine whether they had eggs ready to be fertilized. We obtained fertilization success data by taking the mean number of hatchlings and the mean number of hatchlings plus eggs laid by the two females mated to each male. The data were analyzed using Mann-Whitney *U* tests.

Metabolic rate

We measured resting metabolic rate (RMR) for 12 unparasitized and 11 parasitized, laboratory-reared male crickets descended from individuals captured in Hawaii. Crickets were housed in 0.5-l plastic containers with shelter and ad libitum food (Fluker's cricket food, cat chow) and water and were housed and tested at 30 ± 1°C. Repeated RMR measurements were taken on these crickets once per day on days 1–5 after infestation of the parasitized crickets.

We used open-flow respirometry to measure rates of CO_2 production ($\dot{V}CO_2$; ml/g/h). Measurements were made using a LiCor 6251 CO_2 analyzer capable of resolving differences of 0.2–0.4 ppm of CO_2 in air. Flow rates of dry, CO_2-free air (100–200 ml/min) were maintained at ±1% by a Tylan mass flow controller. Excurrent air from the chamber was dried (magnesium perchlorate) before entering the LiCor 6251. Outputs from both instruments (as well as ambient temperature measured with thermocouples) were recorded on Macintosh computers equipped with National Instruments A/D converters and custom software for data acquisition and analysis (WartHog Systems, written by M. A. Chappell and available at www.warthog.ucr.edu). We measured RMR in chambers constructed from 0.5-l plastic containers and maintained at 30 ± 1°C in an environmental cabinet. RMRs were determined as the mean minimal steady-state $\dot{V}CO_2$ during periods of at least 10 min when activity (indicated by abrupt changes in $\dot{V}CO_2$) was absent. We calculated $\dot{V}CO_2$ as:

$$\dot{V}CO_2 = \dot{V}(FECO_2 - FICO_2) \div \{1 - FECO_2[1 - (1/RQ)]\}, \quad (1)$$

where $\dot{V}$ is flow rate corrected to standard temperature and pressure (STP; 0°C and 101.3 kPa), $FICO_2$ is the initial fractional concentration of CO_2 (zero in these experiments), $FECO_2$ is the final fractional concentration of CO_2, and RQ is the respiratory quotient (the ratio of CO_2 produced/O_2 consumed). We used an RQ of 0.85, which was previously measured in an independent group of *T. oceanicus* (Chappell, unpublished data). The value of RQ used in Equation 1 had little effect on calculated $\dot{V}CO_2$ because $FECO_2$ was very small (<.002).

We measured maximal metabolic rate (MMR) as $\dot{V}CO_2$ during intense, forced exercise for 8 unparasitized and 11 parasitized wild-caught males from Hilo, Hawaii. These crickets had been captured 3 days before testing and were kept in 0.5-l plastic containers with shelter and ad libitum food (Fluker's cricket food, cat chow) and water. They were housed and test-

ed at 27 ± 1°C. Repeated MMR measurements were taken on days 2, 4, and 6 after infestation.

Because of the field location, we used a simpler closed system to measure MMR. We placed single crickets inside a 140-ml syringe equipped with a stopcock valve and flushed the syringe with dry, CO_2-free air (scrubbed with Dryerite and soda lime). The syringe was then sealed and shaken in a uniform motion for exactly 5 min, forcing the cricket to exercise vigorously. At the conclusion of exercise, the air in the syringe was injected through a small tube of desiccant (magnesium perchlorate) into the CO_2 analyzer, and the maximum CO_2 concentration ($FE CO_2$) was recorded. Since $FI CO_2$ was zero, we computed $\dot{V}CO_2$ as:

$$\dot{V}CO_2 = [FECO_2(140 \text{ ml}/5 \text{ min})] \div \{1 - FECO_2[1 - (1/RQ)]\}, \quad (2)$$

using a value of 0.85 for RQ (as for Equation 1, slight errors in the estimated RQ have little effect on calculated $\dot{V}CO_2$, since $FECO_2$ never exceeded 0.004).

Before analysis, we corrected all metabolic rates to 30.0°C using a Q_{10} of 2.5 (Chappell, unpublished data). In both treatment groups, metabolism varied linearly with mass over the small mass range of the crickets. Accordingly, we corrected for mass effects by dividing metabolic rates by mass and tested for differences between groups with repeated-measures ANOVA (JMP; SAS Institute, 1995).

Validation of $\dot{V}CO_2$ as a metabolic index

We present data for $\dot{V}CO_2$ rather than rates of O_2 consumption ($\dot{V}O_2$) because the sensitivity and stability of the LiCor 6251 CO_2 analyzer are approximately 100-fold better than that of the best available O_2 analyzer. However, using $\dot{V}CO_2$ to measure metabolism requires careful consideration because the energy equivalence of $\dot{V}CO_2$ strongly depends on whether lipid, protein, or carbohydrate is used to fuel respiration, and because released CO_2 may come from buffered storage in body fluids as well as directly from respiration. Therefore, we conducted pilot studies during which both $\dot{V}O_2$ and $\dot{V}CO_2$ were measured during rest and exercise in a low flow-rate, open system, which generated concentration changes in both gases that were large enough for accurate measurements. We used modified 0.5-l plastic containers (for RMR) and 30-ml syringes (for MMR) as metabolic chambers, with flow rates of dry, CO_2-free air of 100–120 ml/min. Excurrent gas was dried using magnesium perchlorate, passed through the LiCor 6251, scrubbed of CO_2 with Ascarite, redried, and passed through an Applied Electrochemistry S-3A oxygen sensor. Changes in gas content were recorded as described above. We calculated $\dot{V}O_2$ as:

$$\dot{V}O_2 = \dot{V}(FIO_2 - FEO_2)/(1 - FIO_2), \quad (3)$$

where FIO_2 is the initial fractional concentration of O_2 (0.2095), and FEO_2 is the final fractional concentration of O_2. We calculated $\dot{V}CO_2$ using Equation 1. We performed validation tests on five crickets at rest and during forced exercise. The RQ during rest was constant over time (mean ± SD = 0.78 ± 0.09) and did not significantly differ between exercise and rest (0.75 and 0.78, respectively; $t = -0.26$, $p = .80$), demonstrating that $\dot{V}CO_2$ is an accurate index of energy expenditure in *T. oceanicus*.

Energy allocation

To determine whether parasitized crickets allocate energy resources differently from unparasitized crickets, we dissected MMR crickets under 40× magnification and obtained dry weights of the wings, head, thoracic exoskeleton, abdominal exoskeleton, legs, thoracic muscles, testes plus accessory glands, and fat body by drying each part to a constant weight in a 55°C drying oven and weighing each to 0.01 mg using a Cahn 21 electrobalance. We removed all parasitoid larvae and associated breathing tubes before weighing.

We also determined the dry weights of a different group of male crickets, each of which was exercised only once, on one of days 1, 2, 3, or 4 after infestation (using the methods described above) and euthanized on the day after exercise. These males were captured in Hilo, Hawaii, 3 days before the first day of testing (day 1: $n = 2$ parasitized and 5 unparasitized, day 2: $n = 2$ parasitized and 6 unparasitized, day 3: $n = 3$ parasitized and 3 unparasitized, day 4: $n = 4$ parasitized and 4 unparasitized).

We combined dry masses to obtain measures of reproductive tissues (testes plus accessory glands plus thoracic muscle) and storage tissue (fat body). We calculated the proportion of resources allocated to each tissue type as: proportion devoted to reproduction = (thoracic muscle mass + testis mass + accessory gland mass)/(head mass + thorax mass + abdomen mass + leg mass + wing mass + thoracic muscle mass + testis mass + accessory gland mass); proportion devoted to storage = fat body mass/(head mass + thorax mass + abdomen mass + leg mass + wing mass + thoracic muscle mass + testis mass + accessory gland mass).

Our measures of dry mass do not account for variation in per-gram energy content of tissues. However, we assume that the relative fractions of lipid and lipid-free mass were not significantly different between comparison groups. Residuals of dry masses were normally distributed (group exercised once: reproductive tissue proportion, $W = 0.96$, $p = .39$, storage tissue proportion, $W = 0.97$, $p = .65$; group exercised repeatedly: reproductive tissue proportion, $W = 0.98$, $p = .99$, storage tissue proportion, $W = 0.98$, $p = .95$), and differences between parasitized and unparasitized males were evaluated using ANOVA (JMP; SAS Institute, 1995).

RESULTS

Calling activity

Males in the infested group ($n = 8$) called significantly more before parasitoid infestation than after infestation (Wilcoxon paired-sample test; mean percentage of the night spent calling before = 50%, mean after = 1%, $T = 0$, $p < .01$; Figure 1). Although males in the control group ($n = 6$) exhibited a slight decrease in calling activity after the second anesthetization, this was not statistically significant (mean before = 37%, mean after = 23%, $n = 6$, $T = 7$, $p > 0.5$; Figure 1).

Spermatophore production

Unparasitized males produced significantly more spermatophores per night than parasitized males (repeated-measures ANOVA; experiment 1: unparasitized mean ± SE = 3.3 ± 1.8, parasitized = 0.5 ± 1.2, $F_{1,24} = 29.78$, $p = .0001$; experiment 2: unparasitized = 1.51 ± 1.50, parasitized = 0.31 ± 0.70, $F_{1,19} = 7.22$, $p = .01$; Figure 2), although the two groups did not differ in body size (pronotum width; experiment 1: $F = 0.05$, $p = .82$; experiment 2: $F = 0.08$; $p = .78$). Males in experiment 1, who were individually isolated before the start of the experiment, produced more spermatophores and peaked in spermatophore production earlier in the night than males in experiment 2. There was no significant variation in spermatophore production across nights (experiment 1: $F = 0.03$, $p = .86$; experiment 2: $F = 0.385$, $p = .77$), but there was a significant night × parasitization status interaction in the first experiment (experiment 1: $F = 5.21$, $p = .03$; experiment 2:

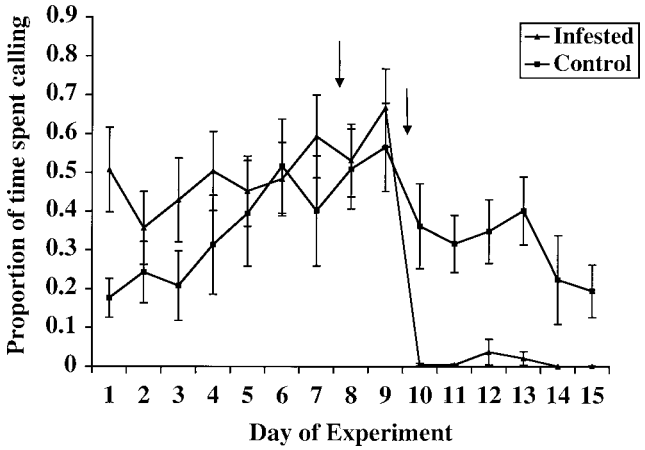

Figure 1
Calling activity of infested (n = 8) and control (n = 6) *T. oceanicus* males. Points show mean proportion of time spent calling and bars indicate SEs. Arrows indicate either anesthesia and handling (between days 7 and 8 for both groups and between days 9 and 10 for control males) or anesthesia and parasitoid infestation (between days 9 and 10 for infested males).

F = 0.71, p = .56) because unparasitized males increased spermatophore production from night 4 to night 6, whereas parasitized males decreased spermatophore production.

Mating activity

Unparasitized males (n = 16) were mounted more and failed at mounting solicitation attempts more than parasitized males (n = 19; Kruskal-Wallis test; mountings: unparasitized mean ± SD = 4.00 ± 2.85, parasitized = 1.10 ± 1.52, Z = 3.41, p = .0007; failures: unparasitized = 1.19 ± 0.75, parasitized = 0.37 ± 0.60, Z = 3.09, p = .002). However, the proportion of attempts that resulted in a successful mounting did not differ between groups (unparasitized mean ± SD = 0.71 ± 0.26, n = 16; parasitized = 0.70 ± 0.39, n = 11, Z = 0.60, p = .54), suggesting that the differences resulted from fewer attempts by parasitized males rather than from rejection of parasitized males by females. As previously described for *T. oceanicus* (Burk, 1983), courtship song always preceded mounting, and males never successfully forced a copulation.

Fertilization success

Unparasitized and parasitized males did not differ in the number of hatchlings they produced (Mann-Whitney U test: unparasitized mean ± SE = 26.42 ± 68.24, parasitized = 27.10 ± 52.73, U = 16, $p > .05$). There was a great deal of variability in hatchling production among females (mean ± SE = 28.10 ± 13.06), with several females producing no hatchlings. The low hatchling production could not be explained by age group (U = 47, $p > .05$) or body size (pronotum width; regression: r^2 = .024, F = 0.46, p = .51). Because the low hatching success may have resulted from environmental conditions in the chamber, unhatched eggs were also counted and a Mann-Whitney U test performed on hatchlings plus eggs laid. The results were identical to the first test, so that there was no difference between unparasitized and parasitized males in either the fertility (hatchling production) or the fecundity (all eggs laid) of the females they were mated with. Dissections revealed that 17 of the 21 females, including most of those that had no hatchlings, retained at least 50 eggs in their ovaries.

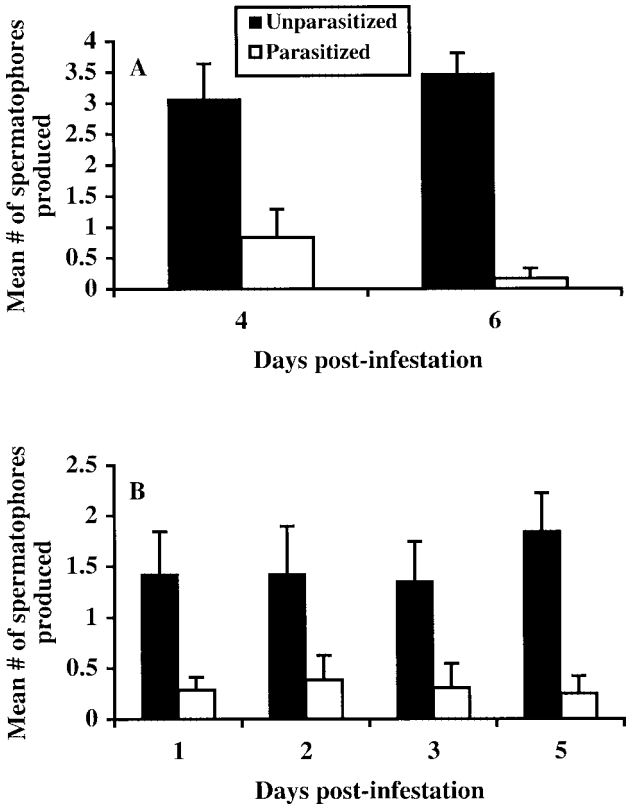

Figure 2
Mean number of spermatophores produced by unparasitized and parasitized *T. oceanicus* males. Results of (A) first and (B) second spermatophore replacement experiment. Bars show SEs.

Metabolic rate

Parasitized and unparasitized crickets did not differ significantly in either resting or maximal metabolic rates (repeated-measures ANOVA; resting: $F_{1,25}$ = 0.21, p = .65; maximal: $F_{1,32}$ = 0.04, p = .85; Figures 3 and 4). However, there was a significant decrease in maximal metabolic rate across days for both treatment groups (resting: $F_{2,21}$ = 1.15, p = .33; maximal: $F_{2,17}$ = 9.80, p = .0005). There was no significant parasite treatment × day interaction for resting or maximal metabolic rates ($p > .10$ for both).

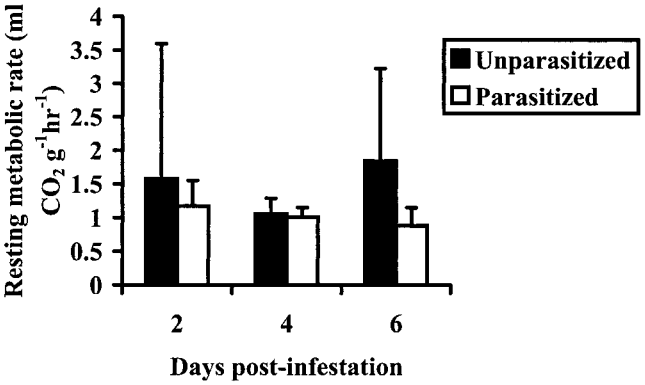

Figure 3
Resting metabolic rates (corrected to 30°C) of parasitized (n = 11) and unparasitized (n = 12) *T. oceanicus* males. Values are mass-specific (using wet mass). Bars show SDs.

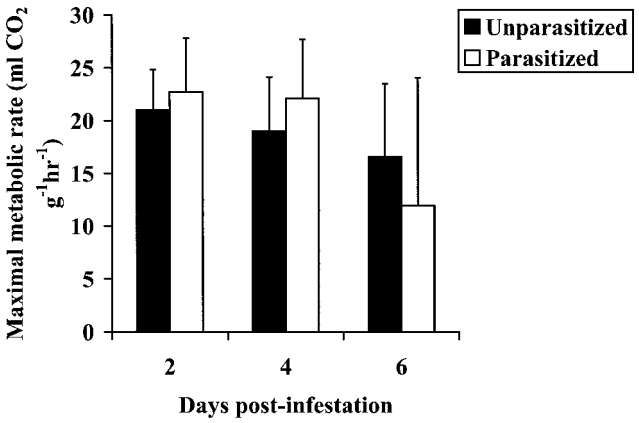

Figure 4
Maximal metabolic rates (corrected to 30°C) of parasitized (n = 8) and unparasitized (n = 11) *T. oceanicus* males exercised once per day on days 2, 4, and 6 post-infestation. Values are mass-specific (using dry mass). Bars show SDs.

Energy allocation

Parasitized males in the MMR group, who were exercised repeatedly and weighed on day 6 after infestation (i.e., late in the course of parasitoid development), had devoted a significantly smaller proportion of the total mass to reproductive tissue (testes + accessory glands + thoracic muscle; unparasitized mean ± SE = 0.068 ± 0.005, parasitized = 0.019 ± 0.004; ANOVA: $F_{1,15}$ = 56.47, $p < .0001$) and storage tissue (fat body; unparasitized mean ± SE = 0.109 ± 0.011, parasitized = 0.064 ± 0.009; ANOVA: $F_{1,15}$ = 10.18, p = .0061) than unparasitized males. However, parasitized and unparasitized males in the other group, who were exercised once and weighed on either day 1, 2, 3, or 4 after infestation, did not differ in the proportion of reproductive or storage tissues (ANOVA: reproductive tissue, $F_{1,17}$ = 0.03, p = .87; storage tissue, $F_{1,17}$ = 0.39, p = .54; Figure 5). There was a significant decline in storage tissue with time ($F_{3,17}$ = 4.13, p = .03) but no significant day × treatment group effect (reproductive tissue: $F_{3,17}$ = 0.61, p = .62; storage tissue: $F_{3,17}$ = 2.47, p = .10). There was no evidence that the proportion of mass devoted to reproduction increased with time in either treatment group (Figure 5).

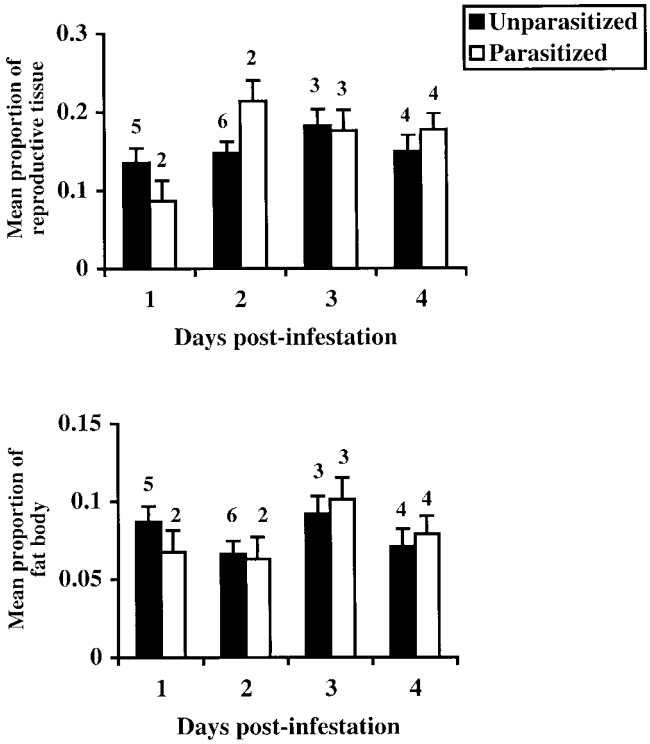

Figure 5
Proportion of dry mass devoted to reproduction (tests + accessory glands + thoracic muscle) and to storage (fat body) by parasitized and unparasitized *T. oceanicus* males exercised once only, on day 1, 2, 3, or 4 post-infestation. Numbers above the bars indicate sample sizes. Bars show SEs.

DISCUSSION

The reproductive compensation hypothesis predicts that parasitized animals should increase reproductive effort to offset losses resulting from their shortened expected life span (Minchella and Loverde, 1981). *Ormia ochracea* larvae do not consume host tissues during the first few days of infestation (Adamo et al., 1995). Parasitized crickets are therefore expected to increase reproductive effort during this time to partially compensate for losses incurred later, when larvae migrate to the abdomen and cause serious injuries to the host (Adamo et al., 1995; Forbes, 1993). Our observations of parasitized *T. oceanicus* crickets contradict this hypothesis. The reproductive effort (spermatophore production, calling, mating activity, and proportion of mass allocated to reproductive tissue) of parasitized males was significantly lower than that of unparasitized males. Calling, spermatophore production, and mating were reduced even during the initial stages of infestation. *O. ochracea* is therefore similar to parasites that castrate their hosts by consuming reproductive tissues, by interfering biochemically with the host, or by limiting host nutrients so that investment into reproduction is impossible (Hurd and Webb, 1997; Kuris, 1974; Reed and Beckage, 1997). *O. ochracea* larvae consume thoracic muscles and fat body (but not reproductive tissue; Adamo et al., 1995). The decrease in fat body points to an energy trade-off as the likely source of reduction in host reproductive effort. However, other effects such as alteration of the hormonal composition of cricket hemolymph (Adamo, 1994) may also be responsible for reduced host reproduction.

Parasitoid-induced decreases in reproductive activity may obscure the detection of an increase in host reproductive effort if only reproductive activity is measured (Forbes, 1996; Perrin et al., 1996). Therefore, we also examined metabolic rates and mass allocation patterns of infested and healthy males. Parasitized males do not spend less time feeding and do not consume less food than healthy males (Adamo et al., 1995; Kolluru, personal observation). When given ad libitum food and water, parasitized males that were exercised repeatedly devoted a significantly smaller proportion of total mass to both reproductive and storage tissues than did healthy males. Contrary to the idea of reproductive compensation, these parasitized males did not increase the proportion of mass allocated to reproductive tissues. Males that were only exercised once, including those examined early during infestation, did not differ from healthy males in energy allocation patterns. These males also did not show any evidence of adaptive increases in energy devoted to reproduction.

Our metabolic rate measurements provided an estimate of the minimal energy expenditure (resting metabolic rate) and maximal capacity for aerobic metabolic power output (maximal metabolic rate) of crickets in each treatment group. To our knowledge, this is the first examination of the effects of a dipteran parasitoid on host metabolism, although studies of

hymenopteran parasitoids have demonstrated decreased host metabolic rate (Alleyne et al., 1997; Rivers and Denlinger, 1994). Surprisingly, we found no significant difference in the resting or maximal metabolic rates of parasitized and unparasitized males, suggesting that there is only a minimal metabolic cost of parasitism. However, parasitoid larvae may account for as much as 30% of the mass of the host–parasitoid complex on the day of emergence. Therefore, it is likely that parasitoid larvae contributed a substantial fraction of the total resting metabolic rate of the host–parasitoid complex (e.g., Alleyne et al., 1997). We measured the CO_2 production of newly emerged *O. ochracea* larvae and found an average $\dot{V}CO_2$ of 1.07 ml/h/g, corresponding to a potentially large fraction of the resting metabolism of the host–parasitoid complex (Figure 4). This implies that parasitized males have lower resting metabolic rates than our data indicate (assuming the metabolic rate of newly-emerged larvae is similar to that of pre-emergence larvae). Therefore, infested males may be unable to increase reproductive effort because of a reduced metabolic capacity for breaking down storage tissue for reallocation to reproduction. Our finding that maximal aerobic power output during forced exercise was not affected by parasitoids seems inconsistent with that hypothesis. However, those measurements concerned very brief bouts of activity and may not reflect parasitoid-induced constraints on metabolic performance over longer intervals that are more relevant to reproductive output.

Our study aimed to determine the impact of parasitoid infestation on optimal host life-history patterns. Some experimental studies addressing this issue have shown that reproductive effort increases as predicted (e.g., Adamo, 1999). However, it is difficult to interpret a lack of increase in reproduction in response to infestation. We used multiple measures of reproductive and somatic energy expenditure to determine the allocation patterns of parasitized *T. oceanicus* males, all of which contradicted the prediction. However, because reproductive effort is extremely difficult to assess directly, our approach cannot exclude with certainty the possibility that increased reproductive effort by parasitized males was masked by declines in reproductive output relative to healthy males.

There are several possible explanations for why parasitized *T. oceanicus* males may be unable to reallocate resources to reproduction (see also Adamo, 1999). The association between the cricket and fly may be too recent for an adaptive host response to have evolved. Although *T. oceanicus* was introduced into Hawaii at least 125 and possibly as many as 1000 years ago (Kevan, 1990; Otte and Alexander, 1983), it is not known when *O. ochracea* reached Hawaii. However, many cricket and fly generations have undoubtedly passed since the two came together in Hawaii, and *T. oceanicus* song characteristics have had sufficient time to evolve in response to the parasitoid (Kolluru, 1999; Rotenberry et al., 1996; Zuk et al., 1993). Alternatively, the encapsulation response may be too general to elicit an increase in reproductive effort (Adamo, 1999; Vinson, 1990). This is supported by Adamo's (1999) finding that bacterial infection, which induces antimicrobial humoral immune responses, caused reproductive compensation, but that neither *O. ochracea* larvae nor Sephadex beads (both of which induce encapsulation) did so.

Our results demonstrate that parasitoid infestation constrains a male cricket's ability to successfully reproduce and suggest that eavesdropping by the fly should lead to adaptations by the cricket to avoid infestation (Zuk and Kolluru, 1998). Both amount of calling and spermatophore production rate are important for male reproductive success (Kolluru, 1999; Sakaluk and Cade, 1980, 1983; Zuk, 1987), and fly infestation therefore represents a significant fitness cost to crickets even before death. However, further studies are needed to establish the effects of the parasitoid under more natural conditions. For example, although wild-caught silent *T. oceanicus* males are more likely to harbor large fly larvae than calling males (Zuk et al., 1995), one field study showed no significant difference in calling activity between parasitized and unparasitized males (Kolluru, 1999). However, this study did not control for cricket age or for intensity or stage of infestation. Our results also differ somewhat from Cade's (1984) study of calling by parasitized crickets. He found a more gradual decrease in calling activity, possibly because his crickets were less heavily infested, or because the association between *O. ochracea* and *T. oceanicus* is more recent than that with his *Gryllus* crickets. Similarly, although reproductive tissue mass measurements and field data (Zuk, unpublished observation) show pronounced degeneration of the seminiferous tubules of parasitized crickets, our data suggest that parasitized males are able to produce viable sperm (see also Adamo et al., 1995). Therefore, parasitized males may experience limited reproductive success if they are able to produce the occasional spermatophore and successfully attract females. However, under field conditions, parasitized males may be unable to successfully compete for access to females or may be selected against by searching females.

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