

Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*)

Gita R. Kolluru, Gregory F. Grether, Eric Dunlop, and Sandra H. South

Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095, USA

Despite the important effects of diet and parasite infection on male reproductive behavior, few studies have simultaneously addressed their influence on intrasexual selection (male–male competition). We examined the synergistic effects of 2 naturally varying environmental factors, lifetime food intake and infection, with the monogenean parasite *Gyrodactylus turnbulli* on the mating tactics and foraging behavior of male guppies (*Poecilia reticulata*). We allowed fish to interact directly with each other during observations and found that unparasitized males won more intermale contests, courted females more frequently, and received positive responses to courtship displays more frequently than males that had been infected. Infected males devoted more time to foraging and less time to courtship and competition than uninfected males, suggesting that they were energetically limited and could not increase reproductive effort despite their reduced expected lifespan. This interpretation was supported by the observation that greater food intake ameliorated the negative effects of parasite infection on courtship effort. Our results have bearing on how natural variation in food availability and parasite prevalence influence geographic variation in reproductive behavior. *Key words*: food availability, foraging behavior, guppy, *Gyrodactylus*, male–male competition, parasite, reproductive effort. [*Behav Ecol* 20:131–137 (2009)]

INTRODUCTION

Male mating success is usually a function of both attracting mates and repelling rivals, and parasite infection can influence both these factors. Relatively few studies have examined the effect of parasites on intrasexual competition, and even fewer have examined the effects of parasites on intersexual and intrasexual interactions simultaneously. Most studies of the effects of parasites on intrasexual competition have shown that parasitized males are less aggressive and win fewer aggressive contests than their healthy counterparts (red grouse, *Lagopus lagopus scoticus*: Fox and Hudson [2001] and Mougeot et al. [2005]; mouse, *Mus musculus*: Gourbal et al. [2002]; Ozark zigzag salamander, *Plethodon angusticlavius*: Maksimowich and Mathis [2000]; and Western fence lizard, *Sceloporus occidentalis*: Schall and Dearing [1987]). However, parasitized males are expected to attempt to compensate for reduced expectation of future reproduction by increasing current reproductive effort, such as becoming more aggressive, and there is some empirical support for this prediction (e.g., theoretical expectation: Kemp [2006]; empirical support: Abbot and Dill [2001]; both reviewed by Kolluru et al. [2002]). Which of these possible effects of parasitism occurs presumably depends on the degree to which the behavior of parasitized males is limited energetically. Likewise, the effect of parasites on the ability of males to attract mates may depend on the degree to which males are able to compensate behaviorally or in other ways to conceal the infection. Although there have been numerous studies of the effects of parasites on male secondary sexual characters, spawned largely by Hamilton and

Zuk (1982), we know relatively little about how parasites influence the behavioral components of male attractiveness. If limited energetically, infected males may be unable to sustain high levels of courtship activity. This could reduce male mating success directly (via female preference for display rate and/or intensity) and indirectly (via scramble competition for mates, such that males that display more frequently obtain more matings). To test these ideas, we examined the synergistic effects of 2 naturally varying factors, food availability and parasite infection, on male mating tactics in the guppy (*Poecilia reticulata*), a tropical freshwater fish.

Guppy populations in Trinidad experience varying levels of food availability as a consequence of variation in canopy openness and resulting algae productivity (algae is the primary food source for guppies in these streams; Grether et al. 1999, 2001). Male guppies can switch among displaying to attract females, sneaking copulations without first displaying, and aggressively usurping access to females via male–male competition (Houde 1997; Kolluru and Grether 2005). Geographic variation in food availability influences aggressive behavior such that males in sites with abundant food pursue the aggressive male–male competition tactic more frequently than males in food-limited areas (Kolluru and Grether 2005; Kolluru et al. 2007).

Sites also differ in the prevalence (proportion of fish infected) of *Gyrodactylus turnbulli* (Lyles 1990; van Oosterhout et al. 2003, 2007), an external monogenean trematode parasite that commonly infects guppies in Trinidad (Lyles 1990). This parasite reproduces directly on fish skin (epidermis) with no intermediate host and is transmitted by direct contact between fish (Scott and Anderson 1984). Parasite loads in the field can be as high as 20 or even 100 parasites per individual guppy (van Oosterhout et al. 2006). Kolluru et al. (2006) showed that parasite populations grew faster on males raised on the higher of 2 food levels, suggesting that well-fed males provide higher resource levels to the parasites. *Gyrodactylus turnbulli* infection reduces the carotenoid content and resulting chroma (color saturation) of male guppy orange spots (Houde and Torio 1992), a primary target of female choice

Address correspondence to G.R. Kolluru, who is now at the Biological Sciences Department, California Polytechnic State University, 1 Grand Avenue, San Luis Obispo, CA 93407, USA. E-mail: gkolluru@calpoly.edu.

Received 11 February 2008; revised 4 September 2008; accepted 6 September 2008.

(Kodric-Brown 1989; Grether 2000). Previous studies have yielded conflicting results with respect to the effects of *G. turnbulli* infection on male guppy courtship display rate (Kennedy et al. 1987; López 1998); however, infected males were less attractive to females (Houde and Torio 1992; López 1998). The studies cited above excluded direct contact among fish by using clear partitions, in order to focus on male display and female choice (Kennedy et al. 1987; Houde and Torio 1992; López 1998). These studies consequently could not examine the effects of parasite infection on behaviors that require close contact between fish, such as sneak copulation, interference competition, and dominance interactions.

Despite intensive study (reviewed by Houde 1997), there is debate with respect to how environmental factors influence male guppy reproductive strategies, most notably male–male competition (reviewed in Houde 1997; Price and Rodd 2006; Kolluru et al. 2007). In the present study, our goal was to simultaneously address 2 important environmental factors, food availability and parasite infection, that may together account for substantial variation in male guppy reproductive behavior. We compared the behavior of males raised on different food levels that had been infected with *G. turnbulli* with males that were sham infected (parasite load data for the same fish were reported in Kolluru et al. 2006). We allowed males to interact directly with each other and with sexually receptive females. Our goals were to determine 1) how parasite infection influences male mating tactics, particularly whether it reduces courtship display and intermale aggression and 2) whether the effects of parasite infection are exacerbated by reduced food availability (i.e., reduced lifetime food intake).

MATERIALS AND METHODS

Experimental design

The overall procedure involved raising males from birth on 1 of 2 food levels, experimentally infecting a random half of the males with *G. turnbulli*, disinfecting the males (i.e., removing all *G. turnbulli* using medication) after 11–12 days to prevent female choice based on the visible parasites and to prevent transmission to other fish during tests, and then carrying out behavioral trials in which pairs of previously infected and control (uninfected) males from the same diet group were observed interacting with 2 virgin females. Behavioral trials were conducted 1–2 days after disinfection. Details are given below.

Study populations

The fish used in this study were first-generation (G_1) laboratory descendants of fish collected from 4 streams (2 drainages, Madamas and Quare) in the Northern Range of Trinidad in June 2000 (see Kolluru and Grether [2005] for site descriptions). All these sites are in undisturbed primary or old secondary forest and can be classified as “low predation” because they contain no predatory fish except *Rivulus hartii*, which only occasionally eats guppies and preys mainly on juveniles (Endler 1978). To maximize the genetic diversity of fish used in the experiment, we obtained offspring for the G_1 generation from approximately 120 (25–35 per population) wild females. This represents a potentially much larger number of sires because females mate multiply in the wild and can store sperm for up to 8 months (Winge 1937).

Food level manipulation

The fish were housed at the University of California, Los Angeles, in a temperature-controlled (24.0 ± 1.5 °C water temperature) room at 12:12 h photoperiod (mixed daylight spectrum fluores-

cent and incandescent light). Water was treated with 2-chloro-4, 6-bis-(ethylamino)-s-triazine (Algae Destroyer; Aquarium Pharmaceuticals, Chalfont, PA), and visible algae were removed regularly. Wild-caught females were individually housed in 8-l tanks, fed a standard diet of commercial flake food (Tetramin and Tetra Spirulina; Tetra, Blacksburg, VA) twice per day (once per day on weekends), and allowed to give birth.

G_1 offspring were randomly assigned at birth to either the low food- or high food-level treatment and housed at densities of 1–6 fish per 8-l tank (see below and Kolluru et al. [2006] for details). Although rearing density and sex ratio can influence male guppy behavior (Price and Rodd 2006), we have shown previously that this range of rearing conditions does not significantly influence the reproductive behavior of males from these populations (Kolluru and Grether 2005). Fish were sexed under a dissecting microscope before sexual maturity, at either 13–15 weeks of age (low food) or 10–12 weeks of age (high food). After sexing, males were housed at densities of 1–4 males per 8-l tank, with one female to allow mating. Four weeks prior to the experimental infections, the fish were redistributed and maintained at densities of 4 fish per 8-l tank (3 males and 1 female) until being used in the mate choice tests. Prior to redistribution, each male's color pattern was sketched to identify individual males. We maximized the range of ages of the 3 males in each tank (mean age range per tank \pm standard error [SE] = 405 ± 11 days), to eliminate age differences between parasite treatment groups.

The fish were fed twice daily (once daily on weekends) using a specially designed feeding device that delivered precise quantities of finely ground flake food to each tank (see Kolluru and Grether [2005] for details). Within each food level treatment, food amounts were adjusted to the age and density of fish in the tank. The high food level was approximately as much as guppies of a given age are willing to eat on the feeding schedule described above (based on the presence of uneaten food in the tanks in pilot studies), and the low food level was one-third of that amount. Comparison of the asymptotic sizes of wild-caught males from low-predation, low food- and high food-availability sites with males from the laboratory food treatments demonstrated that the low food level is on the low end of the range that guppies typically experience in the wild and the high food level is in the middle of the range (Kolluru et al. 2006).

Experimental infection and disinfection

The parasite colony was founded using wild-caught infected fish from the upper Quare drainage and the Paria River in Trinidad and maintained as described in Kolluru et al. (2006). The parasite was identified as *G. turnbulli* by J. Cable (Cardiff University School of Biosciences). Experimental infection was carried out according to previously established methods (Lyles 1990; Kolluru et al. 2006). Donor fish (with >100 active parasites) and recipient fish were sedated using MS-222, and 3–5 parasites were allowed to move from the donor to the recipient. Males in the control group ($N = 106$) were anesthetized, sham infected, and handled at the same time as males in the experimentally infected group ($N = 100$).

We anesthetized and checked the fish for parasites under a dissecting microscope (18 \times) on days 3 and 9 postinfection. Control males were anesthetized and handled similarly. On days 11 and 12 postinfection, all males were disinfected using Life Bearer (Aquarium Products, Glen Burnie, MD; 0,0-dimethyl 1-hydroxy 2-trichloromethyl phosphonate). Males were checked after disinfection to ensure that no *G. turnbulli* were present. Males without active parasite infections were used in behavioral trials 13 or 14 days postinfection (i.e., 1 or 2 days postdisinfection).

Focal male observations

We used an open-aquarium design in which the fish could interact directly. Observations were conducted under daylight spectrum fluorescent lighting in 180-l aquaria with multicolored gravel. To minimize competition for food during observations, we fed the fish to satiation twice per observation day and regularly removed algae from the observation aquaria. This allowed us to examine the effects of lifetime food intake without the potentially confounding effects of short-term hunger. After each set of observations, we filtered the water in the aquaria using a high-flow rate charcoal canister filter (MarineLand Magnum 350, Moorpark, CA), to minimize chemical effects on behavior in subsequent observations. To avoid artificially inflating male–male aggression, we used an even sex ratio (2:2) and low densities of fish per observation tank (Houde 1997). We also used males that had not been housed together. Although this can increase aggression levels (Price and Rodd 2006), it minimized the chances that males had already formed dominance relationships that may have influenced our observations. We minimized body size disparities within male and female pairs. Male pairs consisted of one previously infected male and one control (uninfected) male no more than 14 days apart in age and from the same population and diet treatment. Female pairs consisted of 2 mature virgins from the same population as the males. The females were housed out of sight of mature males so that their behavior could not be influenced by prior experience with mature males.

Behavior observations began within 2 h after the lights came on and were concluded within 5 h after the lights came on. A trial was initiated by releasing the 2 males chosen for testing into an observation aquarium, after their color patterns were sketched for individual recognition. Females were released into the aquarium shortly after the males, and then the fish were fed to satiation. Males and females were left together for one night before observations began because virgin females tend to copulate indiscriminately immediately after being placed with males and then are not receptive for a 30-min period after mating, making observations difficult. Because females continue to be receptive to males for several days, it is preferable to observe their mate preferences after a delay; similar delays were used by Houde and Endler (1990) and Grether (2000). On the morning after they were first put together, the fish were fed to satiation again and the first observation session began 15–30 min later. We carried out at least three 5-min focal observations per male, alternating between males in a predetermined, random order, with ≥ 20 min between consecutive focal observations on a given male. Additional focal observations were made on that day if a male did not perform courtship displays in at least 2 of the initial 3 replicates. Immediately after their observation session, males were weighed to the nearest 0.1 mg, and their standard length (distance between the lower jaw and the caudal peduncle) was measured using digital calipers (± 0.01 mm readout).

We recorded the following male behaviors: time spent foraging and following females, rates of sigmoid courtship displays, and rates of sneak copulations (forced copulation attempts not preceded by display, in which gonopodial contact with the female's ventral surface was visible). For male–male interactions, we determined instigation rates and win rates (proportion of interactions won) for 2 types of aggressive interactions: competition (interference with a male already courting a female) and dominance (supplanting, displaying, chasing, or biting directed from one male to another while neither was following or courting a female). We defined the winner for competition as the male who took or kept the female from the other male and for dominance as the male who displayed

the most aggression during the encounter. Most dominance interactions were distinctly one sided, and thus, one male could be classified as the winner and the other the loser. We recorded the responses of females to male courtship displays, using a 5-point scale (1 = no response, 2 = female looks at the displaying male, 3 = swimming toward the male, 4 = “gliding” [characteristic movement of female toward a displaying male that correlates with the likelihood of mating; Houde 1997], and 5 = copulation). Gliding to a nondisplaying male was exceedingly rare and was not scored. We measured male attractiveness in 2 ways (following Houde and Endler 1990; Grether 2000): the proportion of courtship displays that elicited gliding (attractiveness measure 1) and the mean response level (based on the 5-point scale described above) of females to courtship displays (attractiveness measure 2).

Data analysis

Data from all focal observations for a given male were summed prior to analysis. All data were transformed to meet parametric assumptions, and analyses were performed using JMP 5.1 (SAS Institute, Cary, NC). We constructed an analysis of covariance model with parasite treatment and food level as fixed-effects terms, male pair (the pair of males tested together) and site of origin as random-effects terms, and body length (standard length immediately after observations) as a covariate. Male pair was nested within site of origin and food level. All the fixed effects were fully crossed with each other; however, our model had no interactions involving the random effects (male pair, site of origin). Food level was excluded from the competition and dominance win rate analyses because males from different food level groups were never tested together (win rates are only relevant within male pairs).

RESULTS

None of the fish in the control tanks had parasites at either scoring date. Infected males that had no parasites at either of the scoring dates (6 of 106 infected males) were excluded from all subsequent analyses. Infected males had mean \pm SE parasite loads of 9.9 ± 1.2 on day 3 postinfection and 21.3 ± 2.3 on day 9 postinfection. Males in the low-food group and the high-food group had mean standard lengths of 13.96 ± 0.15 and 15.29 ± 0.15 mm, respectively, at the time when behavior observations were conducted.

Aggressive interactions between males

Gyrodactylus turnbulli infection appeared to increase aggression levels but reduce win rates. Parasitized males instigated competition interactions more frequently than unparasitized males (untransformed means \pm SE: unparasitized 0.001 ± 0.0002 , parasitized 0.002 ± 0.0002 ; $F_{1,89} = 4.30$, $P = 0.041$), but unparasitized males won a greater proportion of competition interactions than parasitized males (unparasitized 0.65 ± 0.06 , parasitized 0.40 ± 0.07 ; $F_{1,49} = 7.10$, $P = 0.011$). High-food males instigated competitions more frequently than low-food males ($F_{1,96} = 8.57$, $P = 0.004$), indicating greater aggressive activity when 2 high-food males were together than when 2 low-food males were together. None of the other terms in these models was significant (all $P > 0.07$).

Courtship and sneak copulations

The parasite and food treatments had independent and synergistic effects on male courtship behavior. Unparasitized males spent more time following females than did parasitized males, and high-food males spent more time following females than

Table 1

Results of analysis of variance of male guppy courtship behavior, with food level and parasite treatment as fixed effects, site of origin and male pair as random effects, and body length as a covariate

	Time spent following females	Courtship display rate	Time spent foraging
Parasite treatment	25.40_{1,96}; <0.0001	11.55_{1,96}; 0.001	6.98_{1,96}; 0.010
Food level	31.18_{1,96}; <0.0001	19.41_{1,96}; <0.0001	5.63_{1,96}; 0.02
Parasite treatment × food level	0.67 _{1,96} ; 0.42	5.41_{1,96}; 0.022	0.58 _{1,96} ; 0.45
Site of origin	2.37 _{3,96} ; 0.08	0.75 _{2,96} ; 0.52	1.63 _{3,96} ; 0.19
Body length	3.59 _{1,96} ; 0.06	0.99 _{1,96} ; 0.32	1.93 _{1,96} ; 0.17
Male pair (food level, site of origin)	1.46_{82,96}; 0.04	1.96_{83,96}; 0.00	1.75_{82,96}; 0.004

Male pair is nested within site of origin and food level. Values are F degrees of freedom; P . Statistically significant results ($P < 0.05$) are given in bold.

did low-food males (Table 1; Figure 1A). The same was true for courtship display rate, and for this dependent variable, there was also a significant, positive, food level × parasite treatment interaction (Table 1; Figure 1B). We infer from this interaction that parasitized males were more energy-limited than unparasitized males.

We also found evidence for genetic differentiation among populations in mating tactics. Males from the 2 low-resource-availability sites (Madamas Tributary and Large Crayfish River) engaged in sneak copulations less frequently than males from the 2 high-resource-availability sites (Aqui River and Small Crayfish River; $F_{3,96} = 3.73$, $P = 0.013$). None of the other terms in these models was significant (all $P > 0.062$).

Male attractiveness

Unparasitized males received more glide responses from females per second of observation than parasitized males ($F_{1,96} = 7.92$, $P = 0.006$), and high-food males received more

glides than low-food males ($F_{1,96} = 11.12$, $P = 0.001$; Figure 1C). For attractiveness measure 1 (proportion of displays eliciting a glide response), the only significant term in the model was male pair ($F_{65,60} = 2.02$, $P = 0.004$). The remaining terms in this model and all terms in the model for attractiveness measure 2 (mean response to displays) were not significant (all $P > 0.12$). We observed only 4 successful copulations (i.e., copulation followed by the jerking motion by the male that characterizes sperm transfer; Houde 1997): 2 involved unparasitized males and 2 involved parasitized males, all raised on the high food level.

We used a multiple regression approach to evaluate which aspects of male performance directly influenced female choice. First, to identify putative predictors, we examined correlations between male attractiveness and several male performance measures: time spent following females, courtship display rate, competition win rate, sneak copulation rate, and dominance win rate. After correcting for multiple tests (Bonferroni alpha level for 10 tests = 0.005), all these variables were significantly correlated with at least one attractiveness

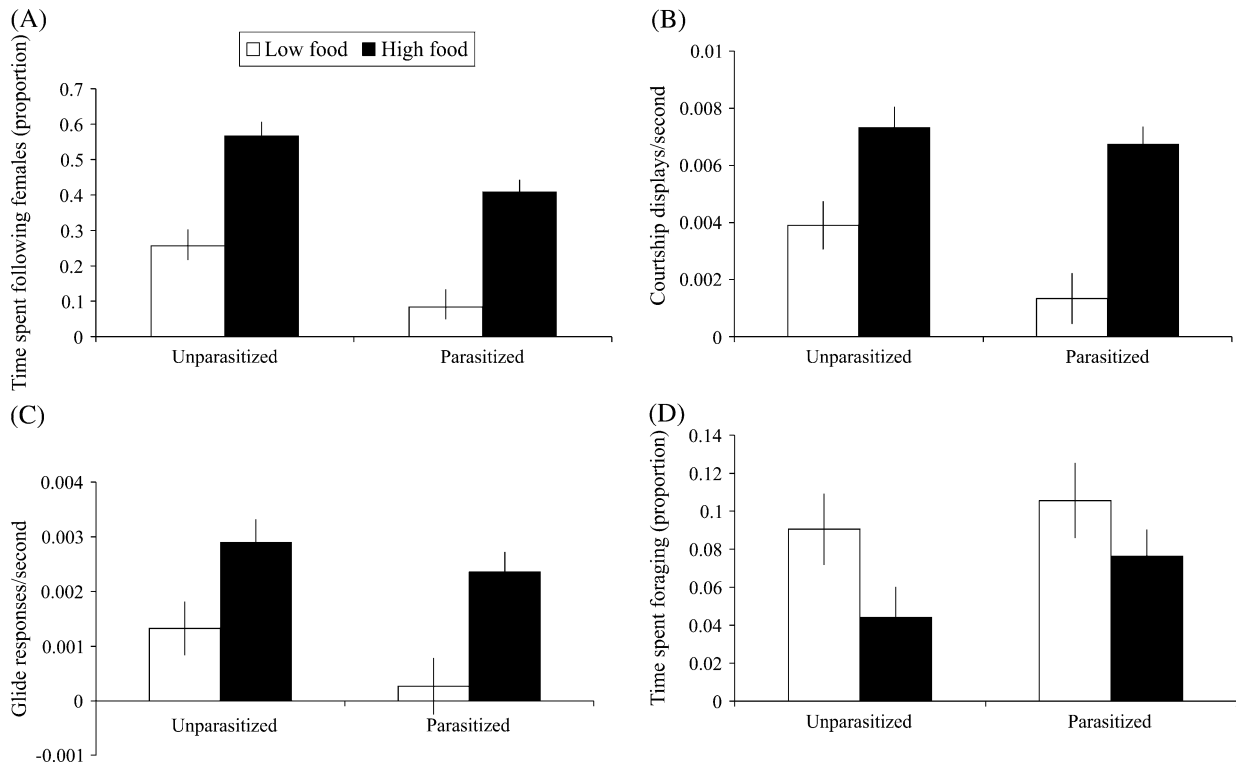


Figure 1 Time spent following females (panel A), courtship display rate (panel B), glide response rate by females (panel C), and time spent foraging (panel D) as a function of parasite treatment and food level. Bars show mean ± 1 SE.

measure ($N = 110\text{--}202$, all $r > 0.22$, all $P < 0.002$) and also with each other (all $r > 0.33$, $P < 0.0003$, with the exception of sneak copulations versus competition and dominance win rates, which had $P > 0.11$). We then constructed 2 multiple regression models (one for each attractiveness measure), with male attractiveness as the response variable and the putative predictors and standard length as independent variables. These analyses revealed that the positive correlation between attractiveness to females and competition win rate was driven by a correlation with time spent following females (attractiveness measure 1: $F_{1,116} = 14.79$, $P = 0.0002$, standardized beta = 0.53; attractiveness measure 2: $F_{1,114} = 27.70$, $P < 0.0001$, standardized beta = 0.67). Courtship display rate was significantly negatively associated with attractiveness measure 2 ($F_{1,114} = 5.79$, $P = 0.018$, standardized beta = -0.27). None of the other terms, including standard length, was significantly associated with attractiveness to females (all $P > 0.06$). We infer that males that were preferred by females followed females more and that these males also won more competitive interactions, but that females did not directly prefer males that won more competitive interactions.

Foraging

Parasitized males spent more time foraging than unparasitized males, and low-food males spent more time foraging than high-food males (Table 1; Figure 1D).

DISCUSSION

Gyrodactylus turnbulli infection had negative effects on the reproductive success of male guppies through both intrasexual and intersexual routes. The effects of infection mirrored those of food limitation and were partially ameliorated by high food intake, which suggests that parasitized males are energetically challenged. As previously reported (Kennedy et al. 1987; Houde and Torio 1992; López 1998), parasitized males courted females less frequently than unparasitized males. Females were equally receptive to parasitized and unparasitized males, but unparasitized males received more positive responses overall simply because they displayed more often. By allowing fish to interact directly, our study also revealed a previously unknown cost to infection in this system: unparasitized males won more intermale contests than males that had been infected. Males who lose aggressive intrasexual contests have reduced mating success (Kodric-Brown 1992, 1993), so this effect compounds the direct negative effects on courtship success. Because we disinfected males immediately prior to testing, our results are potentially conservative with respect to the effects of *G. turnbulli* on male behavior and female response; presumably, these effects would be more pronounced if males harbored parasites, which are likely visible to other fish, at the time of testing.

Parasitized males attempted to aggressively “steal” females from unparasitized males more often than vice versa, but unparasitized males usually won these competitions. Increased aggression with parasitism has been demonstrated in other taxa (e.g., Klein et al. 2004; Cramer and Cameron 2007) and may be the consequence of infected males resorting to costly aggressive tactics to gain access to females who would otherwise be dominated by unparasitized males. In our study, parasitized males could always have courted a female that was not already being courted (because we used an even sex ratio in the mating trials), but it is possible that the most desirable females were monopolized by the unparasitized males (or that parasitized males were not always aware that a second female was present). Alternatively, increased intermale aggression by parasitized males may represent an adaptive increase in mating

effort, a pathological effect of the infection, or a parasite adaptation favoring transmission to new hosts (*Gyrodactylus* is directly transmitted during contact between fish [Poulin 1995; Cable et al. 2002]).

Behavioral variation among guppy populations has been well studied from the perspective of predation intensity (reviewed by Endler 1995; Houde 1997). Recent attention has turned to factors such as food availability (Kolluru and Grether 2005; Kolluru et al. 2007), water velocity (Nicoletto and Kodric-Brown 1999), and parasite prevalence (van Oosterhout et al. 2003, 2007; Martin and Johnsen 2007). Kolluru et al. (2007) examined phenotypic variation in reproductive behavior among 10 low-predation guppy populations in the wild in Trinidad to address whether phenotypic variation among populations differing in food availability represents phenotypic plasticity or genetic divergence (see also Carroll and Corneli 1999; Weitere et al. 2004). Although males were more aggressive in high-food-availability sites, food availability did not account for all the phenotypic variation in reproductive behavior within and among sites. The present results suggest that geographic variation in *Gyrodactylus* prevalence (see Lyles 1990; van Oosterhout et al. 2003, 2006; Martin and Johnsen 2007) could be contributing to variation in reproductive behavior and, thus, provides an example of how geographic variation in behavior can result from multiple environmental factors (Foster and Endler 1999). In low-resource-availability guppy sites in Trinidad, carotenoid availability is low (Grether et al. 1999), which may adversely affect resistance to *G. turnbulli* (Kolluru et al. 2006), and food availability is low (Grether et al. 2001), which causes poorer body condition (mass divided by the cube of standard length), reduced innate resistance to the parasite (Kolluru et al. 2006), and less aggression among males (Kolluru and Grether 2005; Kolluru et al. 2007). Although simultaneous geographic variation in food availability and parasite prevalence may occur in a variety of species (e.g., Chapman et al. 2006), few studies have addressed how food availability or parasite prevalence influence the geographic distribution of reproductive behavior (exceptions include Zuk et al. 1993; Hamilton and Poulin 1999; Kolluru 1999). Our results emphasize that these factors should be examined in the guppy system, especially because *Gyrodactylus* prevalence and food availability both vary among some low-predation sites (Kolluru GR, Grether GF, unpublished data; Grether et al. 2001). In addition, our data suggest that parasite-mediated sexual selection may operate via male-male competition, even in species with strong female mate preference favoring healthy males.

The reproductive compensation hypothesis predicts that parasitized males should increase reproductive effort to compensate for their expected reduced lifespan (reviewed in Kolluru et al. 2002; Schultz et al. 2006). However, the positive effects of food availability on courtship rate suggest that courtship is energetically expensive. We were able to address the possibility that energetic constraints prevented parasitized males from increasing reproductive activity by examining both foraging and courtship activity. Based on our observation that parasitized males foraged more but courted and competed less than healthy males, it is clear that parasitized males did not forego foraging in favor of mating effort; however, as with most studies of reproductive compensation, increases in reproductive effort may have been masked by parasite-induced reduction in reproductive activity (reviewed in Kolluru et al. 2002). Kolluru et al. (2006) showed that the low-food males used in this study were in poorer condition than the high-food males and that the parasitized males were in poorer condition than the control males. Although the parasite loads in our study did not cause obvious illness, the reduction in condition probably caused parasitized males to perform

energetically costly behaviors less frequently than unparasitized males. In support of the idea that *Gyrodactylus* adversely affects body condition in the wild, a field study showed that even small numbers (20 or fewer) of *Gyrodactylus* dramatically increase guppy mortality during floods (van Oosterhout et al. 2007). Furthermore, the detrimental effects of parasite infection on courtship display rate were ameliorated when males were raised on the high food level in the present study, supporting the idea that energetic constraints prevented the infected males from displaying more frequently.

These results suggest that males in high-food-availability sites can engage in energetically costly behavior despite harboring parasites, which has implications for sexual selection for parasite avoidance. Females in such sites are predicted to choose males based on morphological characteristics such as color patterns, indicative of carotenoid intake (Grether 2000), as well as parasite infection (Houde and Torio 1992), rather than courtship display rate. This process can maintain multiple traits for mate choice such that different traits are the best indicators of male quality in different environments (Candolin 2003).

In contrast to previous studies employing similar parasite loads (Houde and Torio 1992; López 1998), we did not find a negative effect of parasite infection on attractiveness to females (proportion of displays that elicited a glide response and mean female response to displays). This may be a function of the population of fish used (both of the cited studies used males descended from the Paria River, which is in a different drainage than the populations used in this study). Alternatively, females may be less able to exert choice when males are being aggressive to each other because courtship displays are often interrupted (Hibler and Houde 2006). Aggressive interference among males occurs frequently in the wild (Kolluru et al. 2007). In addition, parasitized males may have timed their displays for maximal effect or used cues from females to enhance the success rate of their displays, despite displaying less frequently. This type of modulation of courtship behavior has been suggested for guppies in past studies (Houde 1988) and has recently been demonstrated in bowerbirds. Male satin bowerbirds (*Ptilonorhynchus violaceus*) modify the intensity of courtship displays to avoid startling females, thereby increasing the chances of mating (Patricelli et al. 2006).

In summary, we have shown that infection with *G. turnbulli* influences male mating tactics, causing males to invest more effort into foraging and less into courtship. These effects were at least partly exacerbated by reduced food availability, especially in the case of courtship display rate. Our results are consistent with studies showing that unparasitized males win more aggressive contests than infected males (e.g., Fox and Hudson 2001; Schall and Dearing 1987). Increased instigation of aggression by parasitized males also conforms to theoretical predictions that parasitized males are expected to compensate for reduced expectation of future reproduction by increasing current reproductive effort (Kemp 2006). It remains to be determined whether the heightened aggression of parasitized males is adaptive.

FUNDING

National Science Foundation (IBN-0130893 to G.F.G. and G.R.K.).

We thank 2 anonymous reviewers for their comments on the manuscript, A. Carapiet, A. Cardinali, and L. Liu for assistance with parasite infections, J. Cable for identifying the parasite, and Ocean Star International (Snowville, UT), for producing and donating the experimental diets. In Trinidad, we thank the Sinanan family for housing

accommodations, the Ministry of Food Production, Marine Exploitation, Forestry, and the Environment for permits to collect guppies, and the Water and Sewage Authority for permission to work in the upper Quare drainage.

REFERENCES

- Abbot P, Dill LM. 2001. Sexually transmitted parasites and sexual selection in the milkweed leaf beetle, *Labidomera clivicollis*. *Oikos*. 92:91–100.
- Cable J, Scott ECG, Tinsley RC, Harris PD. 2002. Behavior favoring transmission in the viviparous monogenean *Gyrodactylus turnbulli*. *J Parasitol*. 88:183–184.
- Candolin U. 2003. The use of multiple cues in mate choice. *Biol Rev*. 78:575–595.
- Carroll SP, Corneli PS. 1999. The evolution of behavioral norms of reaction as a problem in ecological genetics: theory, methods, and data. In: Foster SA, Endler JA, editors. *Geographic variation in behavior*. New York: Oxford University Press. p. 52–68.
- Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes MJ, Saj TL, Ziegler TE. 2006. Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *Am J Phys Anthropol*. 131:525–534.
- Cramer MJ, Cameron GN. 2007. Effects of bot fly, *Cuterebra fontinella*, parasitism on male aggression and female choice in *Peromyscus leucopus*. *Anim Behav*. 74:1419–1427.
- Endler JA. 1978. A predator's view of animal color patterns. *Evol Biol*. 11:319–364.
- Endler JA. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol Evol*. 10:22–29.
- Foster SA, Endler JA. 1999. Thoughts on geographic variation in behavior. In: Foster SA, Endler JA, editors. *Geographic variation in behavior*. New York: Oxford University Press.
- Fox A, Hudson PJ. 2001. Parasites reduce territorial behaviour in red grouse (*Lagopus lagopus scoticus*). *Ecol Lett*. 4:139–143.
- Gourbal BEF, Lacroix A, Gabrion C. 2002. Behavioural dominance and *Taenia crassiceps* parasitism in BALB/c male mice. *Parasitol Res*. 88:912–917.
- Grether GF. 2000. Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution*. 54:1712–1724.
- Grether GF, Hudon J, Millie DF. 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc R Soc Lond B Biol Sci*. 266:1317–1322.
- Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*. 82:1546–1559.
- Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science*. 218:384–387.
- Hamilton WJ, Poulin R. 1999. Female preference and male nuptial coloration in the freshwater fish *Gobionomorphus breviceps*: geographic variation among populations. *Can J Zool*. 77:463–469.
- Hibler TL, Houde AE. 2006. The effect of visual obstructions on the sexual behaviour of guppies: the importance of privacy. *Anim Behav*. 72:959–964.
- Houde AE. 1988. The effects of female choice and male-male competition on the mating success of male guppies. *Anim Behav*. 36: 888–896.
- Houde AE. 1997. Sex, color, and mate choice in guppies. Princeton: Princeton University Press.
- Houde AE, Endler JA. 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science*. 248:1405–1408.
- Houde AE, Torio A. 1992. Effect of parasitic infection on male colour pattern and female choice in guppies. *Behav Ecol*. 3:346–351.
- Kemp DJ. 2006. Ageing, reproductive value, and the evolution of lifetime fighting behaviour. *Biol J Linn Soc Lond*. 88:565–578.
- Kennedy CEJ, Endler JA, Poynton SL, McMinn H. 1987. Parasite load predicts mate choice in guppies. *Behav Ecol Sociobiol*. 21:291–295.
- Klein SL, Zink MC, Glass GE. 2004. Seoul virus infection increases aggressive behaviour in male Norway rats. *Anim Behav*. 67:421–429.
- Kodric-Brown A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav Ecol Sociobiol*. 25:393–401.

- Kodric-Brown A. 1992. Male dominance can enhance mating success in guppies. *Anim Behav.* 44:165–167.
- Kodric-Brown A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. *Behav Ecol Sociobiol.* 32:415–420.
- Kolluru GR. 1999. Variation and repeatability of calling behavior in crickets subject to a phonotactic parasitoid fly. *J Insect Behav.* 12: 619–626.
- Kolluru GR, Grether GF. 2005. The effects of resource availability on alternative mating tactics in guppies (*Poecilia reticulata*). *Behav Ecol.* 16:294–300.
- Kolluru GR, Grether GF, Contreras H. 2007. Environmental and genetic influences on mating strategies along a replicated food availability gradient in guppies (*Poecilia reticulata*). *Behav Ecol Sociobiol.* 61:689–701.
- Kolluru GR, Grether GF, South SH, Dunlop E, Cardinali A, Liu L, Carapiet A. 2006. The effects of carotenoid and food availability on resistance to a naturally occurring parasite (*Gyrodactylus turnbulli*) in guppies (*Poecilia reticulata*). *Biol J Linn Soc.* 89:301–309.
- Kolluru GR, Zuk M, Chappell MA. 2002. Reduced reproductive effort in male field crickets infested with parasitoid fly larvae. *Behav Ecol.* 13:607–614.
- López S. 1998. Acquired resistance affects male sexual display and female choice in guppies. *Proc R Soc Lond B Biol Sci.* 265:717–723.
- Lyles AM. 1990. Genetic variation and susceptibility to parasites: *Poecilia reticulata* infected with *Gyrodactylus turnbulli* [PhD thesis]. Princeton: Princeton University.
- Maksimowich DS, Mathis A. 2000. Parasitized salamanders are inferior competitors for territories and food resources. *Ethology.* 106:319–329.
- Martin CH, Johnsen S. 2007. A field test of the Hamilton-Zuk hypothesis in the Trinidadian guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol.* 61:1897–1909.
- Mougeot F, Evans SA, Redpath SM. 2005. Interactions between population processes in a cyclic species: parasites reduce autumn territorial behaviour of male red grouse. *Oecologia.* 144:289–298.
- Nicoletto PF, Kodric-Brown A. 1999. The relationship among swimming performance, courtship behavior, and carotenoid pigmentation of guppies in four rivers of Trinidad. *Env Biol Fish.* 55:227–235.
- Patricelli GL, Coleman SW, Borgia G. 2006. Male satin bowerbirds, *Ptilonorhynchus violaceus*, adjust their display intensity in response to female startling: an experiment with robotic females. *Anim Behav.* 71:49–59.
- Poulin R. 1995. “Adaptive” changes in the behaviour of parasitized animals: a critical review. *Int J Parasitol.* 25:1371–1383.
- Price AC, Rodd FE. 2006. The effect of social environment on male-male competition in guppies (*Poecilia reticulata*). *Ethology.* 112:22–32.
- Schall JJ, Dearing MD. 1987. Malarial parasitism and male competition for mates in the western fence lizard, *Sceloporus occidentalis*. *Oecologia.* 73:389–392.
- Schultz ET, Topper M, Heins DC. 2006. Decreased reproductive investment of female threespine stickleback *Gasterosteus aculeatus* infected with the cestode *Schistocephalus solidus*: parasite adaptation, host adaptation, or side effect? *Oikos.* 114:303–310.
- Scott ME, Anderson RM. 1984. The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology.* 89:159–194.
- van Oosterhout C, Harris PD, Cable J. 2003. Marked variation in parasite resistance between two wild populations of the Trinidadian guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Biol J Linn Soc.* 79:645–651.
- van Oosterhout C, Joyce DA, Cummings SM, Blais J, Barson NJ, Ramnarine IW, Mohammed RS, Persad N, Cable J. 2006. Balancing selection, random genetic drift, and genetic variation at the major histocompatibility complex in two wild populations of guppies (*Poecilia reticulata*). *Evolution.* 60:2562–2574.
- van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable C. 2007. Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int J Parasitol.* 37:805–812.
- Weitere M, Tautz D, Neumann D, Steinfartz S. 2004. Adaptive divergence vs. environmental plasticity: tracing local genetic adaptation of metamorphosis traits in salamanders. *Mol Ecol.* 13:1665–1677.
- Winge O. 1937. Succession of broods in *Lebistes*. *Nature.* 140:467.
- Zuk M, Simmons LW, Cupp L. 1993. Calling characteristics of parasitized and unparasitized populations of the field cricket *Teleogryllus oceanicus*. *Behav Ecol Sociobiol.* 33:339–343.