Biological Journal of the Linnean Society, 2006, **89**, 301–309. With 4 figures

The effects of carotenoid and food availability on resistance to a naturally occurring parasite (*Gyrodactylus turnbulli*) in guppies (*Poecilia reticulata*)

GITA R. KOLLURU*, GREGORY F. GRETHER, SANDRA H. SOUTH, ERIC DUNLOP, ANDREA CARDINALI, LINDA LIU and ANDREH CARAPIET

Department of Ecology and Evolutionary Biology, University of California, 621 Charles E. Young Drive South, Los Angeles, CA 90095-1606, USA

Received 19 April 2005; accepted for publication 5 December 2005

Dietary carotenoids have been shown to confer immunological benefits to some species of animals in which males also use these pigments to attract mates. Thus, the potential exists for an allocation trade-off between the sexual and immunological functions of carotenoids. Food availability may also influence immune system function. The present study examined the effects of carotenoid and food availability on the resistance of male guppies (*Poecilia reticulata* Peters) from four wild populations to the parasite *Gyrodactylus turnbulli* Harris. Intermediate levels of carotenoid ingestion resulted in the lowest parasite loads, which suggests that carotenoids strengthen parasite resistance at low levels but either benefit parasites or suppress host immunity at high levels. Males raised on the high-food level initially had fewer parasites, suggesting heightened innate immunity relative to males raised on the low-food level. Over the course of the experiment, however, the high-food males supported higher parasite population growth rates than the low-food males. The results obtained emphasize the importance of evaluating the effects of diet on multiple aspects of immune system function, and caution against assuming that positive effects of carotenoids on immunity in one context will automatically translate to other contexts.

ADDITIONAL KEYWORDS: geographical variation – parasite load – parasite resistance – susceptibility – trade-off.

INTRODUCTION

In many species of fish and birds, males produce brilliant carotenoid-based skin and feather colours to attract females (Olson & Owens, 1998; Møller *et al*., 2000). Evidence is mounting that carotenoids are important immuno-stimulating agents in these species (Blount *et al*., 2003; McGraw & Ardia, 2003; Grether *et al*., 2004). Carotenoids cannot be synthesized by animals, and must be obtained from the diet (Goodwin, 1984); therefore, males may face a trade-off between allocating carotenoids to sexual displays vs. combating parasite infection (Lozano, 1994). The immune response to parasite infection is also energetically costly because it diverts resources from other fitness-enhancing activities, such as reproduction, and is therefore expected to be influenced by host energy intake (Munger & Karasov, 1989; Siva-Jothy & Thompson, 2002). To our knowledge, the relative effects of carotenoid and food intake on parasite resistance have not previously been examined in any species.

Male guppies display carotenoid-based sexual coloration that is absent in females (Hudon, Grether, & Millie, 2003) and female preference for high-carotenoid males is well established (Kodric-Brown, 1989; Houde, 1997; Grether, 2000). Guppies have also been the subject of intensive studies of parasitism (Scott & Anderson, 1984; Kennedy *et al*., 1987; Houde & Torio, 1992; Richards & Chubb, 1996) and, to a more limited extent, the link between carotenoids and immunity (López, 1998; Grether *et al*., 2004). Guppies obtain carotenoids primarily from attached unicellular algae, which is also the main food source for these fish (Dussault & Kramer, 1981). Algae availability limits

*Corresponding author. E-mail: gkolluru@obee.ucla.edu

carotenoid deposition in the orange spots of male guppies in the wild (Grether, Hudon & Millie, 1999) and has also been shown to influence growth rates, life history traits, and behaviour (Grether *et al*., 2001; Reznick, Butler, & Rodd, 2001; Kolluru & Grether, 2005). Dietary carotenoids have been shown to enhance the foreign tissue (allograft) rejection response in male, but not female guppies, which suggests that males face a trade-off between allocating carotenoids to immune function vs. mate attraction (Grether *et al*., 2004). Allografts are not, of course, an immunological challenge that fish encounter in nature. The primary objective of this study was to determine whether carotenoid availability enhances the resistance of guppies to an ectoparasite that they encounter commonly in the wild, *Gyrodactylus turnbulli*.

Gyrodactylus species are monogenean trematodes that infect a variety of fish (Harris & Lyles, 1992; Bakke, Soleng & Harris, 1999; Bakke, Harris, & Cable, 2002). These viviparous flatworms reproduce directly on the skin (epidermis) with no intermediate host, and transmission among host individuals is either by direct contact between fish or via the water column or substrate (Scott & Anderson, 1984; Soleng, Jansen & Bakke, 1999). Under laboratory conditions, the generation time is approximately 2 days and every individual is born with a pregnant embryo in its uterus; thus, parasite population growth rates can be very high (Scott, 1982; Lyles, 1990). In the field, infected guppies usually harbour one to five parasites, but parasite loads greater than ten are sometimes seen (Lyles, 1990; G. R. Kolluru & G. F. Grether unpubl. data). Infected male guppies exhibit reduced carotenoid coloration (Houde & Torio, 1992), courtship display rate (Kennedy *et al*., 1987; López, 1998), and feeding activity (Van Oosterhout, Harris, & Cable, 2003). Intense infections can be fatal (Scott & Anderson, 1984; Lyles, 1990), but fish can combat *Gyrodactylus* infection via substances in their skin mucus (Harris, Soleng & Bakke, 1998; Jones, 2001; Bakke *et al*., 2002; Holland & Lambris, 2002). Guppies exhibit innate and acquired resistance to *Gyrodactylus* (Richards & Chubb, 1996; López, 1998). Acquired resistance is heritable (Madhavi & Anderson, 1985; Bakke *et al*., 1999), although environmental factors such as immunosuppressive chemicals (Dalgaard, Nielsen, & Buchmann, 2003) and stress (Harris, Soleng & Bakke, 2000) can reduce resistance. Resistance to *Gyrodactylus* also varies among host populations (Cable, Harris, & Bakke, 2000; Hedrick, Kim, & Parker, 2001; Soleng & Bakke, 2001; Dalgaard *et al*., 2003), including guppy populations in Trinidad (Van Oosterhout *et al*., 2003).

To examine the long-term, developmental effects of carotenoid and food availability on the resistance of male guppies to *Gyrodactylus turnbulli*, and to test for population differences in these effects, a common garden experiment was conducted in which male guppies from four sites in Trinidad were raised from birth on two different food levels and three different dietary carotenoid concentrations. The reason for manipulating life-long access to food and carotenoids, as opposed to short-term access, was to simulate the conditions that guppies from populations differing in resource availability experience in nature (Grether *et al*., 2001; Reznick *et al*., 2001). Because food availability and the carotenoid concentration of the food were manipulated independently, it was possible to distinguish between the independent effects of these variables.

MATERIAL AND METHODS

Study populations

We studied first-generation (G_1) laboratory-reared descendants of fish collected in 2000 from eight to ten pools from each of four sites in the Northern Range of Trinidad (for grid references, see below). All four sites are classified as 'low predation' sites because they contain no fish predators of guppies except the weak predator *Rivulus hartii* (Houde, 1997). To maximize the genetic diversity of fish used in the experiment, offspring were obtained 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). The sites occur in intact primary or old secondary growth rainforest, with relatively homogeneous forest canopy cover within each site, and are separated from sites differing in predator assemblage by multiple barriers to guppy dispersal, including two or more waterfalls (Grether *et al*., 2001). One stream was chosen with relatively low canopy openness, and another with relatively high canopy openness, in each of two phylogenetically distinct river drainages. This sampling design helps control for phylogenetic effects to the extent that populations within one drainage are closer to each other genetically than populations in different drainages, as would be expected from the dispersal mode of these fish. Previous work has shown that, in streams with lower canopy openness, guppies ingest algae at lower rates and males have less carotenoid pigments in their orange spots (Grether *et al*., 1999, 2001). The names, resource availability levels, and GPS co-ordinates (Universal Transverse Mercator Grid, Zone 20) of the sites are: Aqui River (high resource; PS 939 887) and a tributary of the Madamas River (low resource; PS 950 880) in the upper Madamas drainage; Small Crayfish River (high resource; PS 965 835) and Large Crayfish River (low resource; PS 965 832) in the upper Quare drainage.

Food level and carotenoid diet manipulation

The laboratory populations were housed at the University of California, Los Angeles campus, in a temperature-controlled (24.0 ± 1.5 °C water temperature) room under a 12 : 12 h photoperiod (mixed daylight spectrum fluorescent and incandescent light). To prevent the fish from eating algae, the water was treated with 2-chloro-4,6-bis-(ethylamino)-s-triazine (Algae Destroyer, Aquarium Pharmaceuticals), and visible algae was removed regularly. Wild-caught females were individually housed in 8-litre tanks, fed a standard diet of commercial flake food (Tetramin and Tetra Spirulina) 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- or high-food level treatment, and to either the trace, low- or high-carotenoid diet treatment. Prior to sexing, the fish were housed in 8-litre tanks in mixed-sex broods at densities of one to six fish per tank. Each tank potentially contained offspring from multiple females, but offspring did not vary in age by more than 14 days. Fish were sexed under a dissecting microscope well before sexual maturity, at either 13–15 weeks of age (low food) or 10–12 weeks of age (high food), when sedated with MS-222. Black pigment spots near the gonopore (females) and skin iridescence or the beginnings of gonopodial development in the anal fin (males) were identified. After sexing, males were housed at densities of one to four males per tank, with one companion female to allow mating. From 4 weeks before the start of the experiment until the completion, all males were maintained at densities of four fish per tank (three males and one companion female). To avoid accidental differences in age among treatment groups, the range of ages of the three males in each housing tank were maximized (mean ± SE age range per tank: 405 ± 11 days). The experiment involved 174 infected males and 152 control males. Prior to being moved into the experimental tanks, the unique colour pattern of each male was sketched to identify the three males in each tank.

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. 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. As the fish aged, food amounts were augmented weekly by 10.8% (high-food treatment) and 12.6% (low-food treatment), over the first 20 weeks. Because male guppies essentially stop growing after reaching sexual maturity (Snelson, 1989), food levels were not increased after 20 weeks of age.

The carotenoid diets (trace, low and high) were designed to contain different concentrations of the carotenoid pigments found in the natural diets of guppies, but otherwise were identical. The basal (trace-carotenoid) diet comprised spray-dried white fish meal (41.8%), wheat flour (47.0%), vegetable oil (2.0%), vitamin premix (1.0%), and gelatine (8.1%). The estimated protein (40%) and fat content (10%) of this diet are similar to high-quality commercial fish feeds for tropical fish. The vitamin premix included vitamin A palminate, but no carotenoids. Lutein and β-carotene in gelatine beadlet form were added to the low- and high-carotenoid diets (Roche Vitamins Inc.); the amount of pure gelatine added to these diets was adjusted to keep protein content constant across diets. Based on high-performance liquid chromatography (HPLC) analyses of diet samples, the mean composition of the low-carotenoid diet was 2.85 μg g^{-1} lutein, 0.21 μg g^{-1} zeaxanthin, and 1.99 μg g^{-1} β-carotene (5.05 μg g^{-1} total carotenoids), and the mean composition of the high-carotenoid diet was 745.57 μg g^{-1} lutein, 71.43 μg g^{-1} zeaxanthin, and 522.30 μg g^{-1} β-carotene (1339.30 μg g^{-1} total carotenoids; D. F. Millie, pers. comm.). The total carotenoid content of the trace carotenoid diet was negligible (≤ 0.2 μg g^{-1}), but the fish apparently obtained small amounts of carotenoids from algae, despite efforts to eliminate algae growth (see Results). By design, the carotenoid content of the high-carotenoid diet was similar to what guppies could obtain by consuming pure unicellular algae, whereas the carotenoid contents of the trace- and low-carotenoid diets were well below this range. For example, green algae (Chlorophyta) is in the range 250–2280 μg g^{-1} lutein, 50–1020 μg g^{-1} zeaxanthin, and 260–820 μg g^{-1} β-carotene (for these and other algal carotenoid content data, see Goodwin, 1980: chapter 7). The carotenoid diets were custom made and donated by Ocean Star International, Inc (Burlingame, CA).

Pigment analyses

To evaluate how the experimental diets compared to the diets of guppies in the wild in terms of the amounts of carotenoids actually deposited in the skin of the fish, the total skin carotenoid content of 72 males raised on the experimental diets (these males were not infected with *G. turnbulli*) was quantified. The total skin carotenoid content of each male was divided by body weight, to yield a body size-adjusted measure of carotenoid content. Mean values for each carotenoid diet group were compared with published data on wild-caught fish from six sites in Trinidad (Hudon *et al*., 2003).

Prior to the pigment extractions, the fish were sedated with MS-222, frozen instantly in liquid nitrogen and stored at –80 °C. To extract the pigments, the skin was thawed at room temperature, peeled off the body with surgical instruments, and allowed to dry for a few minutes. Carotenoids were extracted with acetone, transferred to a new vial, concentrated under a flow of nitrogen to remove the acetone, and re-dissolved in hexane. Absorption spectra of the hexane extracts were measured with an Ocean Optics USB-2000 spectrometer equipped with a cuvette holder and a deuterium-tungsten light source (Ocean Optics DT-1000).

Experimental infection

To establish a laboratory colony of *Gyrodactylus*, 60 infected fish were transported from each of two sites, the Upper Quare drainage and the Paria River (a site not included in this study, but known to harbour *G. turnbulli*; Harris & Lyles, 1992), to the laboratory. The parasite colony was maintained by housing infected guppies at high densities (> 40 fish per 40-litre tank) and adding three to five uninfected fish each week (Scott & Anderson, 1984; Lyles, 1990). The parasite was identified as *G. turnbulli* by J. Cable (Cardiff University School of Biosciences) based on 16 specimens from the parasite colony.

Experimental infection was carried out according to previously established methods (Lyles, 1990). Donor fish (with > 100 parasites) and recipient fish were sedated and positioned so that the tail of the recipient was lying on top of a heavily infected area on the donor. Subsequently, there was a delay until three to five parasites were seen to move from the donor to the recipient, which usually happened within 10 s. This range of parasite loads was allowed because it was difficult to transfer exactly four parasites per recipient. Occasionally, five parasites moved onto the recipient immediately, and it was impossible to remove them once they attached to the recipient; at other times, it took longer for parasites to move onto the recipient, and waiting for more than three parasites to move would have resulted in the fish and parasites being sedated for too long (over-sedation can kill the parasites and the fish). Males in the control treatment were sedated, sham-infected, and handled at the same time as males in the experimental treatment. The wild-caught donor fish may have harboured other parasites; however, no other parasites were observed moving from hosts to recipients during the infection process, and neither the fish nor the water from the parasite colony came into contact with the experiment fish at any other time.

The parasite load of each male was determined on days 3 and 9 post infection, by sedating the fish and counting the number and position of *G. turnbulli* under a dissecting microscope at ×18 magnification. Control males were sedated and handled similarly. None of the fish in the control tanks had parasites at either of the two scoring dates. Several of the infected fish had no parasites at either of the scoring dates, and were excluded from analyses. On the day prior to infection and on day 13 post infection, all males were anaesthetized and weighed to the nearest 0.1 mg and their standard length (the distance between the lower jaw and the caudal peduncle) was measured using digital callipers with a 0.01 mm readout.

Data analysis

Parasite load (a measure of parasite resistance) is defined here as the number of parasites per infected host individual. To evaluate the influence of site, food level and carotenoid diet on parasite load, a repeated measures analysis of variance (ANOVA) model was constructed with parasite loads at 3 and 9 days post infection as the dependent variables, time as the repeated factor, and site, food level, and carotenoid diet as nonrepeated fixed effects. This model also included a random effects tank term nested within site, carotenoid diet, and food level, to take into account the common environment shared by males within a tank. Initially, male age was included in the model; however, there was no significant effect of age on parasite loads ($F_{1,100} = 1.74$, $P = 0.19$), and age was therefore excluded. The rate of increase of parasite load is a measure of the parasite population growth rates on fish in the treatment groups (Lyles, 1990; Van Oosterhout *et al.*, 2003). To evaluate whether diet influenced parasite population growth rates, interactions between diet and time were examined.

Mass divided by the cube of standard length was used as an index of condition; this is a standard isometric condition index (Jones, Petrell & Pauly, 1999; Grether, 2000), and the results were essentially the same when an allometric index was used (data not shown). Differences in male condition at the two scoring dates were evaluated using ANOVA with parasite treatment, site, carotenoid diet and food level as the main effects. The four-way interactions in these models were not significant (both $P > 0.38$) and were excluded from the final models. All data were transformed to meet parametric assumptions, and all analyses were conducted using JMP 3.2.2 (SAS Institute, Inc.), at an alpha level of 0.05.

RESULTS

Diet in the laboratory versus the field

To evaluate how the food levels used in this experiment compare to what guppies typically experience in the field, the standard lengths at maturity of

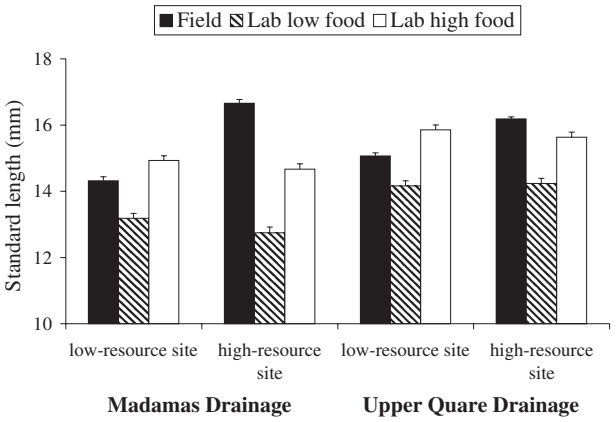

Figure 1. Standard lengths of mature male guppies from field-caught and laboratory-reared samples. Bars show the least squares mean + SE (except where the standard error is too small to be shown).

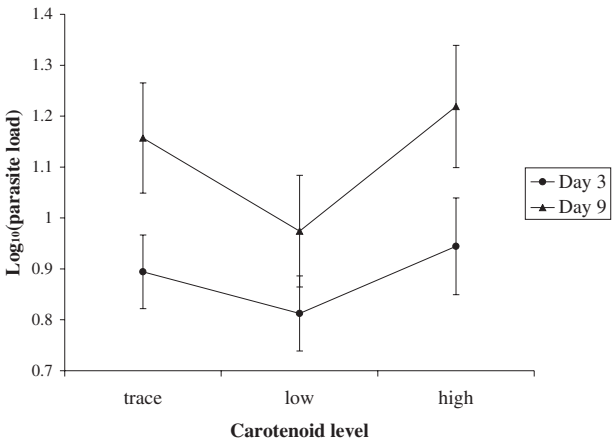

Figure 2. Parasite loads as a function of carotenoid intake at 3 and 9 days post infection. Points indicate the least squares mean ± SE.

laboratory-reared and field-caught males were compared. On average, males raised on the high-food level were significantly larger than wild-caught males from low-resource streams ($t_{177} = 1.97$, $P = 0.005$; Fig. 1) but smaller than wild-caught males from high-resource streams ($t_{146} = 1.98$, $P < 0.0001$). By contrast, males raised on the low-food level were significantly smaller than the wild-caught males from low-resource streams ($t_{196} = 1.97$, $P < 0.0001$); nevertheless, the ranges of standard lengths in these two groups overlapped broadly (low-food laboratory-reared: 11.03–16.11 mm; low-resource wild-caught: 12.67–19.28 mm). These results suggest that the high-food level was in the middle of the range that guppies typically experience in the wild whereas the low-food level was on the low end of the range.

Judging from the total carotenoid content of the skin of males raised on the experimental diets, the trace-carotenoid diet was substantially lower in carotenoids (mean ± SE: 4.61 ± 0.44 ng of carotenoids per mg of body weight) than that encountered by guppies in nature (range of six population means: 6.80–14.70 ng mg^{-1}; Hudon *et al*., 2003) whereas the low- and high-carotenoid diets (8.04 ± 0.87 ng mg^{-1} and 11.48 ± 1.18 ng mg^{-1}, respectively) were within the natural range.

EFFECTS OF DIET AND SITE ON PARASITE LOADS

There was a significant difference in parasite loads among the carotenoid diet groups (Table 1; Fig. 2). Low-carotenoid males had the lowest parasite loads, followed by trace-carotenoid males. The highest parasite loads occurred in the high-carotenoid males. Independent contrasts indicated that the parasite loads of males in the trace- and high-carotenoid groups were indistinguishable from each other ($F_{1,103} = 0.87$, $P = 0.35$), but that each differed significantly from the low-carotenoid group (trace vs. low: $F_{1,103} = 4.93$, $P = 0.029$; low vs. high: $F_{1,103} = 8.02$, $P = 0.006$).

There was no significant difference in absolute parasite load between the low- and high-food level groups (Table 1; Fig. 3A). At 3 days post infection, low-food males had the highest parasite loads, but the effects of food level were reversed by 9 days post infection, as reflected by a significant time × food level interaction (Table 1). In addition, mass-specific parasite loads (parasite loads divided by the starting mass) were examined. Qualitatively, these analyses yielded the same results as those reported above, except that there was a significant food level effect caused by the greater mass-specific loads for low-food males at 3 days post infection (Fig. 3B). However, the difference between the food groups disappeared by 9 days post infection (Fig. 3B).

Parasite loads and the increase in parasite loads with time varied with the site of origin of the fish (Table 1; mean ± SE number of parasites per fish 3 days post infection: Madamas drainage high resource 0.82 ± 0.11, Madamas drainage low resource 1.00 ± 0.08, Upper Quare drainage high resource 0.87 ± 0.08, Upper Quare drainage low resource 0.85 ± 0.10; 9 days: Madamas drainage high resource 1.16 ± 0.13, Madamas drainage low resource 1.23 ± 0.12, Upper Quare drainage high resource 1.21 ± 0.12, Upper Quare drainage low resource 0.86 ± 0.15). There were significant differences among sites in the influence of carotenoid diet on parasite loads (site × carotenoid diet interaction; Table 1; Fig. 4).

Table 1. Results of the repeated measures analysis of variance evaluating the effects of the nonrepeated factors site, food level, and carotenoid diet, and the repeated factor time, on the parasite loads of male guppies

Effect	*F* (d.f.)	*P*
Between subjects effects		
Tank (site, carotenoid diet, food level)	4.15 (43, 103)	< 0.0001
Site	4.69 (3, 103)	0.004
Carotenoid diet	4.60 (2, 103)	0.012
Food level	0.0005 (1, 103)	0.98
Site × carotenoid diet	5.65 (6, 103)	< 0.0001
Site × food level	2.12 (3, 103)	0.10
Carotenoid diet × food level	2.10 (2, 103)	0.13
Site × carotenoid diet × food level	2.09 (6, 103)	0.06
Within subjects effects		
Time	32.23 (1, 103)	< 0.0001
Time × site	5.16 (3, 103)	0.002
Time × carotenoid diet	1.61 (2, 103)	0.20
Time × food level	13.53 (1, 103)	0.0004
Time × site × carotenoid diet	0.72 (6, 103)	0.64
Time × site × food level	0.73 (3, 103)	0.53
Time × carotenoid diet × food level	0.42 (2, 103)	0.66

The four-way interaction is not shown. d.f., degrees of freedom.

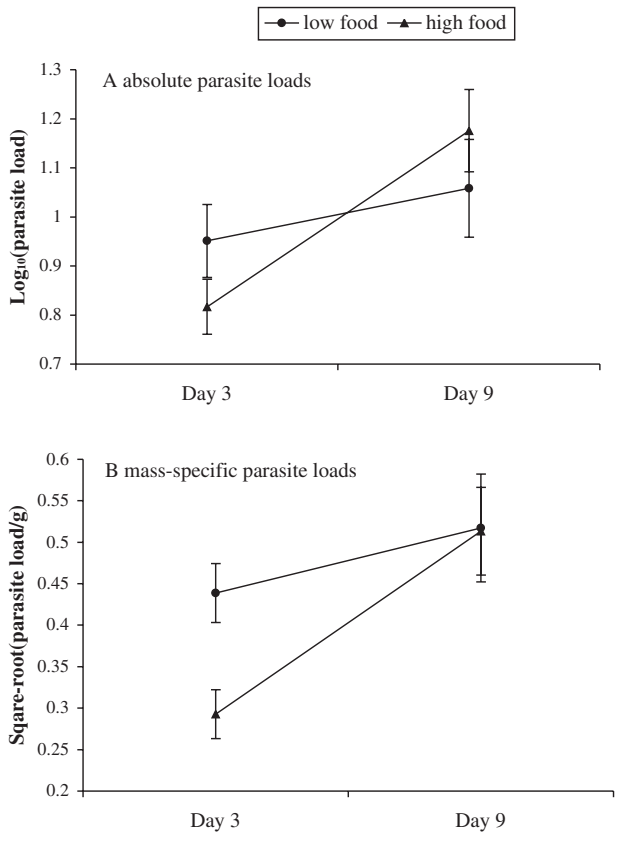

Figure 3. Absolute (A) and mass-specific (B) parasite loads of male guppies at 3 and 9 days post infection. Points indicate the least squares mean ± SE.

Effects of diet and parasite infection on condition

Experimentally infected males did not differ in condition from uninfected control males at 3 days post infection ($F_{1,297} = 0.08$, $P = 0.77$), but were in poorer condition by 9 days ($F_{1,339} = 5.99$, $P = 0.015$). Males from the four sites varied in condition at both scoring times (3 days: $F_{3,297} = 6.39$, $P = 0.0003$; 9 days: $F_{3,339} = 11.06$, $P < 0.0001$), and males in the high-food group were in better condition than males in the low-food group (3 days: $F_{1,297} = 15.30$, $P = 0.0001$; 9 days: $F_{1,339} = 13.90$, $P = 0.0002$). Carotenoid diet had no significant influence on body condition (both $P > 0.13$). There was a significant treatment × diet interaction at day 9 ($F_{2,339} = 4.54$, $P = 0.011$), because condition increased linearly with carotenoid level for experimentally infected males, but was highest at the intermediate carotenoid level for control males. The other terms in the models were not significant ($P > 0.08$).

DISCUSSION

The present common garden experiment revealed significant effects of diet on the resistance of male guppies to *G. turnbulli*. Carotenoid availability had a nonlinear effect on resistance: parasite loads were lowest in males raised on the diet containing an intermediate concentration of carotenoids, whereas the trace- and high-carotenoid diet groups were statistically indistinguishable from each other. Food avail-

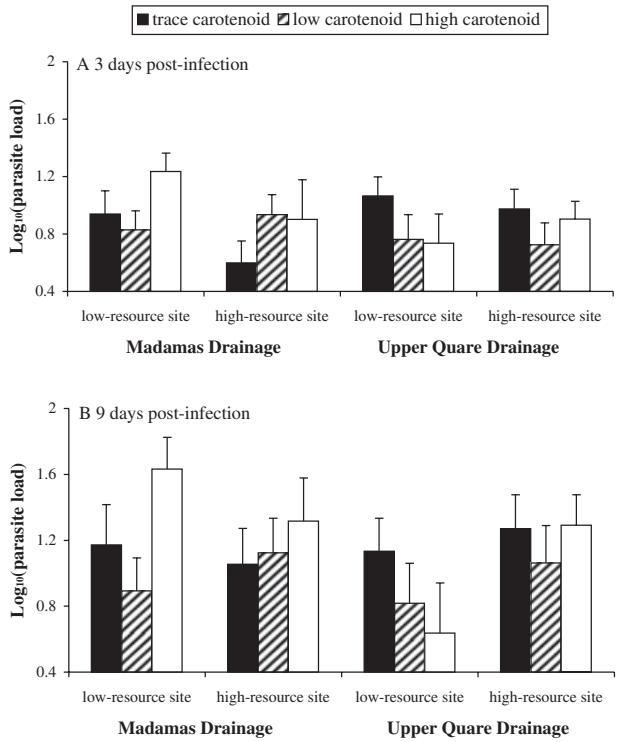

Figure 4. Parasite loads of male guppies infected with *Gyrodactylus* as a function of site and carotenoid diet, at 3 days post infection (A) and 9 days post infection (B). Bars show the least squares mean + SE.

ability had a positive effect on parasite loads at 3 days post infection, suggesting that the innate immunity of males raised on the high-food level was greater than that of males raised on the low-food level. However, this difference disappeared by 9 days post infection, possibly because males raised on the high-food level, who were in better condition, supported greater parasite population growth rates than males raised on the low-food level. Parasite resistance, and the effects of carotenoids on resistance, varied among sites. Because these fish were born and raised in the laboratory, the site differences probably reflect genetic differences in resistance and in the effects of carotenoid intake on resistance. The present experimental design did not, however, enable maternal effects to be ruled out.

Consistent with other studies demonstrating immuno-stimulating properties of carotenoids (Møller *et al*., 2000; Blount *et al*., 2003; McGraw & Ardia, 2003; Grether *et al*., 2004), males in the trace-carotenoid group had higher parasite loads than males in the low-carotenoid group in the present study. However, males in the high-carotenoid group also had high parasite loads and, in some cases, the highest parasite loads, which suggests that high concentrations of carotenoids may reduce parasite resistance (note that the experimental diets contained the same types of carotenoids as found in the algae of Trinidad streams, and that the amount of carotenoids in the high-carotenoid diet was within the natural range for algae; see Materials and Methods). This result contrasts with the previous finding that the high-carotenoid diet used in the present study enhances the foreign tissue rejection response in guppies (Grether *et al*., 2004). The difference may result because high levels of antioxidants, including carotenoids, aid parasites more than they aid hosts (Hõrak *et al*., 2004). Therefore, parasite resistance, unlike tissue rejection, may be reduced under high levels of carotenoid ingestion. These results are analogous to those showing both helpful and harmful effects of carotenoids on human cancer (Omaye *et al*., 1997; Palozza *et al*., 2003; Paolini *et al*., 2003), and highlight the importance of studying a variety of carotenoid dosages and experimental subjects when evaluating the potential health benefits of carotenoids. Whether high concentrations of carotenoids directly benefit parasites, or whether they suppress the host immune system, remains to be determined.

Males raised on the high-food level had lower initial parasite loads than males raised on the low-food level, suggesting that food intake has a positive effect on the innate immune response to *G. turnbulli*. Innate immunity is important if hosts are able to kill the parasites before the parasites reproduce (Jones, 2001; Holland & Lambris, 2002). The idea that energetically-limited males exhibit reduced immunity is consistent with previous studies of the energetic costs of mounting an immune response (Siva-Jothy & Thompson, 2002; Derting & Compton, 2003). However, males raised on the high-food level supported greater parasite population growth rates over the course of the experiment than males raised on the low-food level, perhaps because they had greater energy reserves or skin surface area on which to support the parasites. Reduced food intake results in delayed maturation, reduced growth rates, smaller body size (Reznick, 1990), and reduced aggressive competition for mates (Kolluru & Grether, 2005) in male guppies. It is possible that parasitized males raised on limited food suffer from decreased long-term survival despite having lower parasite loads (Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000; Moret & Schmid-Hempel, 2000; Krist *et al*., 2004), which is an effect that was not measured in the present experiment.

Substantial evidence was found for genetic variation in parasite resistance and in the norm of reaction of parasite resistance to carotenoid intake (i.e. the effect of carotenoid intake on parasite resistance), among the four genetically isolated sites examined. Parasite resistance may evolve in response to a variety of biotic and abiotic factors which may vary among populations (Lyles, 1990; Hamilton & Poulin, 1999; Gleeson,

McCallum, & Owens, 2000; Hedrick *et al*., 2001). Resistance to *Gyrodactylus* is genetically based (Madhavi & Anderson, 1985), and genetic differences in resistance occur among populations of other host species (Leberg & Vrijenhoek, 1994; Dalgaard *et al*., 2003). The sites used in the present study differ with respect to resource availability (Grether *et al*., 2001; Kolluru & Grether, 2005) and parasite prevalence (G. R. Kolluru & G. F. Grether, unpubl. data), and both of these axes of environmental variation may contribute to geographical variation in parasite resistance among these sites. Preliminary laboratory results suggest that males originating from the low resource availability sites support lower *G. turnbulli* population growth rates than males from the high resource availability sites (G. R. Kolluru & G. F. Grether, unpubl. data), which is consistent with the idea that fish that have evolved under conditions of resource scarcity are hardier than fish that have evolved under more benign conditions (for a similar argument, see Van Oosterhout *et al*., 2003). There was also a trend towards greater parasite resistance in males from populations that co-occur with *G. turnbulli* than in males from sites where the parasite is absent. Thus, guppies would be a promising system for further studies on the evolution of parasite resistance.

ACKNOWLEDGEMENTS

We thank Joanne Cable for identifying the parasite, Ocean Star International, Inc., for producing and donating the experimental diets, Roche Vitamins, Inc., for donating carotenoids, and David F. Millie for analysing the carotenoid content of the diets. In Trinidad, we thank the Sinanan family for housing accommodation, 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. This study was supported by National Science Foundation grants IBN-0001309 to G.F.G. and IBN-0130893 to G.F.G. and G.R.K.

REFERENCES

Bakke TA, Harris PD, Cable J. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal of Parasitology* **32:** 281–308.

Bakke TA, Soleng A, Harris PD. 1999. The susceptibility of Atlantic salmon (*Salmo salar* L.) × brown trout (*Salmo trutta* L.) hybrids to *Gyrodactylus salaris* Malmberg and *Gyrodactylus derjavini* Mikailov. *Parasitology* **119:** 467–481.

Blount JD, Metcalfe NB, Birkhead TR, Surai PF. 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300:** 125–127.

Cable J, Harris PD, Bakke TA. 2000. Population growth of *Gyrodactylus salaris* (Monogenea) on Norwegian and Baltic Atlantic salmon (*Salmo salar*) stocks. *Parasitology* **121:** 621–629.

Dalgaard MB, Nielsen CV, Buchmann K. 2003. Comparative susceptibility of two races of *Salmo salar* (Baltic Lule river and Atlantic Conon river strains) to infection with *Gyrodactylus salaris*. *Diseases of Aquatic Organisms* **53:** 173–176.

Derting TL, Compton S. 2003. Immune response, not immune maintenance, is energetically costly in wild white-footed mice (*Peromyscus leucopus*). *Physiological and Biochemical Zoology* **76:** 744–752.

Dussault GV, Kramer DL. 1981. Food and feeding behaviour of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Canadian Journal of Zoology* **59:** 684–701.

Gleeson DJ, McCallum HI, Owens IPF. 2000. Differences in initial and acquired resistance to *Ichthyophthirius multifiliis* between populations of rainbowfish. *Journal of Fish Biology* **57:** 466–475.

Goodwin TW. 1980. *The biochemistry of the carotenoids*, vol. 1. London: Chapman & Hall.

Goodwin TW. 1984. *The biochemistry of the carotenoids*, vol. 2. London: Chapman & Hall.

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. *Proceedings of the Royal Society of London Series B* **266:** 1317–1322.

Grether GF, Kasahara S, Kolluru GR, Cooper EL. 2004. Sex-specific effects of carotenoid intake on the immunological response to allografts in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society of London Series B* **271:** 45–49.

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 WJ, Poulin R. 1999. Female preference and male nuptial colouration in the freshwater fish *Gobiomorphus breviceps*: geographic variation among populations. *Canadian Journal of Zoology* **77:** 463–469.

Harris PD, Lyles AM. 1992. Infections of *Gyrodactylus bullatarudis and Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) in Trinidad. *Journal of Parasitology* **78:** 912–914.

Harris PD, Soleng A, Bakke TA. 1998. Killing of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) mediated by host complement. *Parasitology* **117:** 137–143.

Harris PD, Soleng A, Bakke TA. 2000. Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* **120:** 57–64.

Hedrick PW, Kim TJ, Parker KM. 2001. Parasite resistance and genetic variation in the endangered Gila topminnow. *Animal Conservation* **4:** 103–109.

Holland MCH, Lambris JD. 2002. The complement system in teleosts. *Fish and Shellfish Immunology* **12:** 399–420.

Hõrak P, Surai PF, Ots I, Møller AP. 2004. Fat soluble antioxidants in brood-rearing great tits *Parus major*: relations to health and appearance. *Journal of Avian Biology* **35:** 63–70.

Houde AE. 1997. *Sex, colour, and mate choice in guppies*. Princeton, NY: Princeton University Press.

Houde AE, Torio AJ. 1992. Effect of parasitic infection on male colour pattern and female choice in guppies. *Behavioral Ecology* **3:** 346–351.

Hudon J, Grether GF, Millie DF. 2003. Marginal differentiation between the sexual and general carotenoid pigmentation of guppies (*Poecilia reticulata*) and a possible visual explanation. *Physiological and Biochemical Zoology* **76:** 776–790.

Jones RE, Petrell RJ, Pauly D. 1999. Using modified length-weight relationships to assess the condition of fish. *Aquaculture Engineering* **20:** 261–276.

Jones SRM. 2001. The occurrence and mechanisms of innate immunity against parasites in fish. *Developmental Comparative Immunology* **25:** 841–852.

Kennedy CEJ, Endler JA, Poynton SL, McMinn H. 1987. Parasite load predicts mate choice in guppies. *Behavioral Ecology and Sociobiology* **21:** 291–295.

Kodric-Brown A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behavioral Ecology and Sociobiology* **25:** 393–401.

Kolluru GR, Grether GF. 2005. The effects of resource availability on alternative mating tactics in guppies (*Poecilia reticulata*). *Behavioral Ecology* **16:** 294–300.

Krist AC, Jokela J, Wiehn J, Lively CM. 2004. Effects of host condition on susceptibility to infection, parasite development rate, and parasite transmission in a snail–trematode interaction. *Journal of Evolutionary Biology* **17:** 33–40.

Leberg PL, Vrijenhoek RC. 1994. Variation among desert topminnows in their susceptibility to attack by exotic parasites. *Conservation Biology* **8:** 419–424.

Lochmiller RL, Deerenberg C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88:** 87–98.s.

López S. 1998. Acquired resistance affects male sexual display and female choice in guppies. *Proceedings of the Royal Society of London Series B* **265:** 717–723.

Lozano GA. 1994. Carotenoids, parasites, and sexual selection. *Oikos* **70:** 309–311.

Lyles AM. 1990. Genetic variation and susceptibility to parasites. *Poecilia reticulata* infected with *Gyrodactylus turnbulli*. DPhil Thesis, Princeton University Press.

Madhavi R, Anderson RM. 1985. Variability in the susceptibility of the fish host, *Poecilia reticulata*, to infection with *Gyrodactylus bullatarudis* (Monogenea). *Parasitology* **91:** 531–544.

McGraw KJ, Ardia DR. 2003. Carotenoids, immunocompetence, and the information content of sexual colours: an experimental test. *American Naturalist* **162:** 704–712.

Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poultry and Biology Reviews* **11:** 137–159.

Moret Y, Schmid-Hempel P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290:** 1166–1168.

Munger JC, Karasov WH. 1989. Sublethal parasites and host energy budgets: tapeworm infection in white-footed mice. *Ecology* **70:** 904–921.

Olson VA, Owens IPF. 1998. Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology and Evolution* **13:** 510–514.

Omaye ST, Krinsky NI, Kagan VE, Mayne ST, Liebler DC, Bidlack WR. 1997. β-carotene: friend or foe? *Fundamental and Applied Toxicology* **40:** 163–174.

Palozza P, Serini S, De Nicuolo F, Piccioni E, Calviello G. 2003. Prooxidant effects of β-carotene in cultured cells. *Molecular Aspects of Medicine* **24:** 353–362.

Paolini M, Abdel-Rahman SZ, Sapone A, Pedulli GF, Perocco P, Cantelli-Forti G, Legator MS. 2003. β-carotene: a cancer chemopreventive agent or a co-carcinogen? *Mutation Research* **543:** 195–200.

Reznick DN. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. *Journal of Evolutionary Biology* **3:** 185–203.

Reznick D, Butler IVMJ, Rodd H. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *American Naturalist* **157:** 126–140.

Richards GR, Chubb JC. 1996. Challenge infections, following treatment, of *Gyrodactylus bullatarudis* and *G. turnbulli* (Monogenea) on the guppy (*Poecilia reticulata*). *Parasitology Research* **82:** 242–247.

Scott ME. 1982. Reproductive potential of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* **85:** 217–236.

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.

Sheldon BC, Verhulst S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology and Evolution* **11:** 317–321.

Siva-Jothy MT, Thompson JJW. 2002. Short-term nutrient deprivation affects immune function. *Physiological Entomology* **27:** 206–212.

Snelson FF. 1989. Social and environmental control of life history traits in poeciliid fishes. In: Meffe, GK, Snelson, FF, eds. *Ecology and evolution of livebearing fishes (Poeciliidae)*. Englewood Cliffs, NJ: Prentice Hall, 149–161.

Soleng A, Bakke TA. 2001. The susceptibility of grayling (*Thymallus thymallus*) to experimental infections with the monogenean *Gyrodactylus salaris*. *International Journal of Parasitology* **31:** 793–797.

Soleng A, Jansen PA, Bakke TA. 1999. Transmission of the monogenean *Gyrodactylus salaris*. *Folia Parasitologica.* **46:** 179–184.

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). *Biological Journal of the Linnean Society* **79:** 645–651.

Winge O. 1937. Succession of broods in *Lebistes*. *Nature* **140:** 467.