

SPATIOTEMPORAL VARIATION IN LINEAR NATURAL SELECTION ON BODY COLOR IN WILD GUPPIES (*POECILIA RETICULATA*)

Dylan J. Weese,^{1,2} Swanne P. Gordon,^{3,4} Andrew P. Hendry,^{5,6} and Michael T. Kinnison^{1,7}

¹*School of Biology and Ecology, University of Maine, 5751 Murray Hall, Orono, Maine 04469*

²*E-mail: dylan_weese@umit.maine.edu*

³*Department of Biology, University of California, Spieth Hall, Riverside, California 92521*

⁴*E-mail: swanne.gordon@email.ucr.edu*

⁵*Redpath Museum and Department of Biology, McGill University, 859 Sherbrooke St. W., Montréal, Québec H3A 2K6, Canada*

⁶*E-mail: andrew.hendry@mcgill.ca*

⁷*E-mail: michael_kinnison@umit.maine.edu*

Received June 25, 2008

Accepted December 15, 2009

We conducted 10 mark–recapture experiments in natural populations of Trinidadian guppies to test hypotheses concerning the role of viability selection in geographic patterns of male color variation. Previous work has reported that male guppies are more colorful in low-predation sites than in high-predation sites. This pattern of phenotypic variation has been theorized to reflect differences in the balance between natural (viability) selection that disfavors bright male color (owing to predation) and sexual selection that favors bright color (owing to female choice). Our results support the prediction that male color is disfavored by viability selection in both predation regimes. However, it does not support the prediction that viability selection against male color is weaker in low-predation experiments. Instead, some of the most intense bouts of selection against color occurred in low-predation experiments. Our results illustrate considerable spatiotemporal variation in selection among experiments, but such variation was not generally correlated with local patterns of color diversity. More complex selective interactions, possibly including the indirect effects of predators on variation in mating behavior, as well as other environmental factors, might be required to more fully explain patterns of secondary sexual trait variation in this system.

KEY WORDS: Adaptive divergence, linear selection, mark–recapture, predation, selection differential, selection gradient.

The role of natural selection in generating diversity is frequently inferred from associations between phenotypic variation and environmental features or habitat types (Endler 1986; Schluter 2000). Such evidence for natural selection is indirect because selection itself is not actually quantified (Lande and Arnold 1983; Endler 1986; Kingsolver et al. 2001). Direct estimates of selection in the wild can therefore provide additional insight into adaptive hypotheses by suggesting whether contemporary patterns of se-

lection are consistent with those predicted to produce observed patterns of phenotypic variation (Lande and Arnold 1983; Endler 1986). The best opportunity to witness such selection might often be cases in which trade-offs exist between different components of fitness. This follows from the recognition that although selection might be expected to shift trait values toward adaptive optima, potentially making contemporary selection less apparent, such trade-offs will often prevent phenotypes from being

optimized with respect to any one component of selection (e.g., survival or mating success) (Schluter et al. 1991). Here, we consider natural (i.e., viability) selection on secondary sexual traits, which are generally considered subject to a selective trade-off between natural and sexual selection (Fisher 1930; Endler 1980; Svensson and Gosden 2007). In so doing, we assess the contribution of viability selection to contemporary phenotypic variation in nature.

In addition to the balance between fitness trade-offs, phenotypic evolution will be sensitive to spatiotemporal variation in selection. This variation is likely common in nature, presumably because of fluctuating environmental conditions (reviewed in Siepielski et al. 2009). This spatiotemporal variation in the intensity or direction of selection is commonly proposed as a primary mechanism responsible for the maintenance of trait variation both within and between populations (Barton and Turelli 1989; Merilä et al. 2001; Brooks 2002). Although spatiotemporal variation in natural or sexual selection has been directly documented in some systems (Siepielski et al. 2009), such variation is more commonly indirectly surmised. Importantly, although it is relatively straightforward to test for the statistical significance of any estimate of selection at a given time and place (H_0 : no selection is apparent), such a test is not in itself a statistical evaluation of whether patterns of trait variation are likely the result of variable selection. Rather, the generality of adaptive hypotheses must be statistically assessed by contrasting multiple spatiotemporal estimates of selection (H_0 : selection is spatiotemporally consistent).

Our objectives were to quantify spatiotemporal variation in patterns of natural selection in a classic study system of secondary sexual trait evolution—color patterns of Trinidadian guppies (*Poecilia reticulata*). Using survival data from 10 separate mark–recapture experiments, we estimated linear natural selection coefficients (Lande and Arnold 1983) associated with male coloration (male guppies have colored spots that vary in size and number). Our estimates of natural (viability) selection were then used to evaluate support for current hypotheses for the origin and maintenance of color diversity within and among habitat types.

EVOLUTION OF GUPPY COLOR

Typically, Trinidadian guppy habitats are characterized as either high predation or low predation (Endler 1995). High-predation habitats are usually found in the lower reaches of streams and contain a variety of large, predatory fishes. These predator communities differ somewhat between the south and north slopes of Trinidad's Northern mountain range. The south slope contains a "mainland" community of predators (a subset of the ichthyofauna of South America), whereas the north slope contains a marine-derived "Caribbean" ichthyofauna (Endler 1983). Low-predation

habitats, in contrast, are usually found upstream of barrier waterfalls that have prevented colonization by the above predatory fishes (Endler 1978; Magurran 2005). These low-predation habitats do contain some guppy predators, although these predators are considered less "dangerous." They include a species of killifish (*Rivulus hartii*) on both slopes and several species of predatory prawns (*Macrobrachium* spp.) on the north slope (Endler 1978, 1983; Millar et al. 2006; Gordon et al. 2009; Mckellar et al. 2009). Both *Rivulus* and *Macrobrachium* are also found in high-predation habitats, the latter only on the north slope. Regardless of slope-specific differences in predator communities, the broad contrast between high- and low-predation habitats has been suggested to drive parallel patterns of adaptive divergence in numerous traits, including male color, in many streams (Endler 1978, 1983, 1995; Magurran 2005).

The color patterns of male guppies are influenced by both sexual and natural selection (Endler 1978, 1983). Sexual selection (female mate choice) often (although not always) favors more colorful males (Houde 1987; Endler and Houde 1995; Brooks and Endler 2001). On the other hand, natural selection imposed by predators is expected to favor less conspicuous color patterns (Endler 1978, 1980, 1983; Godin and McDonough 2003; Millar et al. 2006). Broadly consistent with this prediction, males in high-predation guppy populations on both slopes are often (but not always) less colorful than their low-predation counterparts (Endler 1978, 1980, 1983; Alexander et al. 2006; Millar et al. 2006; Karim et al. 2007; Schwartz and Hendry 2007; Kemp et al. 2008). The role of predators in color pattern evolution has been further supported by an introduction of guppies from a high-predation site to a low-predation site, and by multigeneration greenhouse experiments (Endler 1980). In both cases, colored spots were smaller and less numerous in guppies that coexisted with large fish predators compared to those that inhabited control, low-predation treatments, or natural low-predation streams (Endler 1980).

Despite these broadly deterministic patterns, a large amount of local color diversity exists both within and among guppy populations, even within a given predation regime (Endler 1978; Brooks 2002; Millar et al. 2006; Olendorf et al. 2006; Karim et al. 2007). As a result, guppies are commonly regarded as a model system in which to study the factors maintaining variation in adaptive traits. Numerous mechanisms have been advanced as potential explanations (reviewed in Brooks 2002), including frequency-dependent natural selection (Olendorf et al. 2006), frequency-dependent sexual selection (Hughes et al. 1999), local variation in female color preferences (Endler and Houde 1995; Schwartz and Hendry 2007), spatial variation in selection coupled with gene flow (Brooks 2002; Crispo et al. 2006), and temporal variation in selection (Brooks 2002; Gamble et al. 2003). Our study will address the possible contribution of spatiotemporal

variation in natural (viability) selection to the patterns of trait variation.

Despite extensive work on the geographic distribution and evolution of male guppy color, no study has yet directly tested the basic expectation that more conspicuous and colorful guppies face a survival deficit in natural habitats. That is, no studies have actually calculated selection coefficients for color in natural populations of guppies. We suggest that such estimates would be valuable in extending and refining this now classic example of evolution in the wild, and would contribute to a growing body of work emphasizing the biological implications of spatiotemporal variation in natural and sexual selection. Based on previous work in the guppy system, we tested the following hypotheses. (1) Mortality rates are higher in high-predation environments than in low-predation environments (see also Reznick et al. 1996; Gordon et al. 2009). (2) Natural selection generally disfavors more colorful guppies (Endler 1978, 1980), and estimated linear selection coefficients are therefore predominantly negative. (3) The strength of natural selection (selection coefficients) against color is greater in high-predation habitats than in low-predation habitats (Endler 1978, 1980). This prediction is distilled from the general notion, derived from geographic patterns, field introductions, and laboratory evolution, that viability selection against color is more intense (*sensu* Endler 1978) in high-predation habitats. We interpret this notion as predicting that the slope describing the relationship between color and survival should be more strongly negative where guppies coexist with visual-hunting fish predators. (4) Linear selection coefficients and mean trait values should be correlated among populations: populations with less of a given color should experience strong selection against that type of color.

Methods

STUDY SITES

Our study sites were located within three rivers (Marianne, Damier, and Aripo) that flow from Trinidad’s Northern Mountain range (Table S1). The Marianne and Damier rivers drain the north slope, whereas the Aripo River drains the south slope. Additional environmental information about the Marianne River sites (M1, M10, M15, M16, M17), can be found in Crispo et al. (2006) and Millar et al. (2006). The Aripo River sites (AH, AL) are described in Schwartz and Hendry (2007) and the Damier River sites (DH, DL) are those described in Karim et al. (2007) and Gordon et al. (2009). We conducted the majority of our fieldwork during the dry season (March–June) (Table 1)—because flow rates and stream morphology are less variable at this time (Reznick et al. 1996). The sites chosen for our mark–recapture experiments were all characterized by distinct pool-riffle structure. Study sites were typically pools or sets of pools (guppies are rarely found in riffles) selected for features that would minimize emigration (e.g., partial barriers to upstream or downstream movement). In one case, separate mark–recapture experiments were conducted in the same site (Aripo high predation) in two different years (2005 and 2006).

Guppy populations from the Damier River were the result of a 1996 experimental introduction of guppies that originated from the high-predation section of the nearby Yarra River (Karim et al. 2007; Gordon et al. 2009). The Damier selection experiments thus provide a particularly direct test of the hypothesis that colonization of different predation habitats leads to differential selection, because trait values in these populations may not have

Table 1. Summary of mark–recapture information for the 10 experiments. Capture efficiency is the proportion of guppies captured at the first recapture episode (Recap 1), divided by the number known to be alive based on the second recapture episode (Recap 2). Daily mortality rate (Mort rate) is the estimated percentage of the original number released fish that died per day. Killing power (daily exponential mortality rate) is $\log_{10}(N \text{ released})$ minus $\log_{10}(N \text{ at final recap})$ then divided by the duration of the experiment (T). Information for Recap 2 and Capture efficiency are not applicable (n/a) for experiments with only a single recapture event.

Experiment	Release date	<i>N</i> released	Recap 1		Recap 2		Capture efficiency	Mort rate	Killing power
			<i>N</i>	<i>T</i> (days)	<i>N</i>	<i>T</i> (days)			
Low predation									
M16	3/26/2005	65	61	19	45	52	97.97	0.006	0.003
M1	6/29/2004	132	71	11	36	67	90.87	0.011	0.008
M10	5/19/2005	211	147	14	118	30	85.90	0.015	0.008
DL	3/27/2004	87	63	12	n/a	n/a	n/a	0.023	0.012
AL	5/5/2005	95	34	25	n/a	n/a	n/a	0.026	0.006
High predation									
M15	3/28/2004	248	93	13	n/a	n/a	n/a	0.048	0.011
AH05	5/10/2005	100	23	10	n/a	n/a	n/a	0.077	0.014
AH06	4/3/2006	210	79	15	31	44	82.78	0.019	0.019
M17	6/26/2004	111	41	13	21	66	89.76	0.012	0.011
DH	3/28/2004	62	39	12	n/a	n/a	n/a	0.031	0.012

achieved optimum values. All other sites contained indigenous populations.

MARK-RECAPTURE TECHNIQUES

We employed standard mark-recapture techniques for guppies (Rodd and Reznick 1991; Reznick et al. 1996; Bryant and Reznick 2004; Olendorf et al. 2006; Van Oosterhout et al. 2007; Gordon et al. 2009). For each experiment, virtually all of the adult guppies in each pool were captured and transported to our field station in Trinidad. These guppies were kept in aerated tanks that had been treated to prevent fungal infection (Fungus Eliminator, Jungle Laboratories Corporation, Cibolo, TX), reduce stress from handling (Stresscoat, Aquarium Pharmaceuticals, Chalfont, PA), and neutralize toxic chemicals in the water (Amquel, Kordon, Hayward, CA). All guppies were anaesthetized with tricaine methanesulfonate (MS-222), placed on a standard metric grid under full spectrum fluorescent lights (which mimic the daylight spectrum), photographed with a digital camera (Sony MVC-500), and then individually marked with subcutaneous injections of elastomer dye (Northwest Marine Technology, Shaw Island, WA). Using a combination of six different colors and (up to) six different anatomical locations, two subcutaneous injections provided 540 individually identifiable marking codes per sex per experiment. Mortality rate due to tagging was very low (<1%) in the period between tagging and release. Within a few days of tagging, guppies were released back into their site of origin. On several occasions, subsets of marked guppies were retained in the laboratory, verifying that no appreciable delayed mortality resulted from marking.

Recapture episodes occurred at approximately 2-week intervals after the original release date, with some variation (10–14 days) due to field logistics and anticipated mortality rates (e.g., the Aripo low-predation experiment was sampled after 25 days). These are standard and appropriate lengths of time for studies of mortality in adult male guppies because approximately 50% of adult male guppies perish over 2 weeks in high-predation experiments (Reznick et al. 1996; Gordon et al. 2009). Recapture episodes involved intensively sampling each site, where we attempted to catch all guppies. We used butterfly nets, in conjunction with bait stations (wire boxes holding dog food), to capture guppies from particular pools before identifying their marks in the laboratory or field. Very few (<0.1%) guppies lost one of their original marks between marking and recapture. In most of these cases, color patterns recorded in photos (in addition to the single remaining mark) allowed determination of fish identity.

Typically, recapture episodes lasted several days, depending on the number of guppies and the size and complexity of a particular stream site. We stopped fishing when no more guppies were observed, and then returned on one or two subsequent days to capture any remaining guppies. We also sampled upstream and

downstream pools within 300–400 m of our release sites (unless an absolute barrier to guppy movement was present), which prevented potential emigrants from leaving our focal sites. This distance corresponds roughly with the maximum observed movement for male guppies as described by Croft et al. (2003). The vast majority of marked guppies were captured within the focal study areas.

Five experiments (M16, M1, M10, AH06, and M17) included a second recapture episode that targeted individuals surviving the first episode (which had been rereleased following the first recapture episode) (Table 1). For these experiments, we were able to estimate approximate capture efficiency as the number of guppies known to be alive during the first episode (including guppies missed during the first episode but subsequently captured during the second episode) divided by the number of guppies that were captured during the first episode. To compare mortality patterns between regimes, we calculated (for each experiment) the daily mortality rate as the percentage of the original number of guppies released that had died divided by the number of days in each experiment (Begon et al. 1996). To represent the concept that the probability of death can be considered as a rate applied over time, and to account for differences in experimental sample size and duration, we also calculated (for each experiment) the killing power (daily exponential mortality rate) as $\log_{10}(\text{number of fish released}) - \log_{10}(\text{number of fish present at the final recapture episode})$ then divided by the number of days in each experiment (Begon et al. 1996). We used *t*-tests with the different selection experiments as the unit of replication to evaluate regime differences in both daily mortality rates and killing power.

PHOTO ANALYSIS

Using the photographs, each color spot on each guppy was assigned to one of seven color categories (Black, Silver, Orange, Yellow, Green, Blue, Violet). We generally followed the methods of Millar et al. (2006), except that we did not differentiate between fuzzy black and black, or between bronze-green and green. We then measured body area, and the area of each individual color spot with the program ImageJ (Scioncorp.com). No measurements were taken from any fins (including the caudal fin), because such structures are difficult to position in a standard fashion and due to concerns that extra handling time could contribute to mortality. A substantial amount of color can be located on the caudal fin (less so on the dorsal fin); this limitation should be addressed in future work, but does not obviate any inferences with specific respect to selection on body color. We likewise did not take spectroradiometer measurements from each color spot because the required procedure (Kemp et al. 2008) was again considered too stressful for fish being used in a mark-recapture experiment in the wild.

For each individual, the areas of all spots of the same color were summed to obtain the total area of a particular category of

color spot. The colors yellow, silver, and violet were all very rare and so they were not considered individually. They were, however, included in analyses that pooled colors into biologically relevant categories (Endler 1978): structural color (includes violet, silver, and blue spots) and carotenoid color (includes yellow and orange spots). Total color was computed as the entire color spot area on an individual guppy (all color spots pooled).

MEASUREMENT OF NATURAL SELECTION

For experiments with a single recapture episode, captured guppies were assigned an absolute fitness of one, and guppies that were never recaptured were assigned an absolute fitness of zero. In experiments with two recapture episodes, guppies that survived the entire duration of the experiment were assigned an absolute fitness of one, and guppies that survived to the first recapture episode (but not the second) were assigned an absolute fitness proportional to the interval of time between the first and second recapture episode (Brodie and Janzen 1996). Assigning absolute fitness in this manner is suitable for adult male guppies because they attempt to copulate with females continuously following maturity (Magurran 2005), and thus the number of days a male guppy is alive is likely a good surrogate for potential reproductive success. As is customary for direct estimates of natural selection (Lande and Arnold 1983; Brodie et al. 1995), every individual's absolute fitness was converted to relative fitness by dividing by the population mean (independently for each experiment). Trait values were also standardized to a mean of zero and a standard deviation of unity (Lande and Arnold 1983; Brodie et al. 1995), which facilitates the comparison of selection coefficients (Kingsolver et al. 2001).

For experiments with two recapture episodes, simple linear regressions (fitness predicted by a single trait) were used to calculate selection differentials (regression coefficients). Multiple regressions (fitness predicted by all color traits and body area) were used to calculate selection gradients (partial regression coefficients associated with particular traits) (Lande and Arnold 1983; Brodie et al. 1995). Selection differentials indicate total selection (both direct and indirect) acting on a trait, whereas selection gradients estimate selection on a trait while removing the effects of selection on all other measured traits. We did not include total color in the full multiple regression model as this would result in complete collinearity (Mitchell-Olds and Shaw 1987), because total color was determined by adding up all of the individual color elements. We did, however, calculate selection gradients associated with total coloration in a multiple regression that included only body area and total color. In experiments with two recapture events, we assessed temporal variation in selection by calculating selection differentials and gradients separately for each recapture episode. To estimate selection over the second episode, we only considered variation in fitness among the subset of the popula-

tion that survived the first recapture episode. Therefore, for these analyses we implemented the analytical approaches appropriate for studies with a single recapture episode (described below). Episode-specific estimates of selection necessarily have reduced sample size and experimental duration; therefore, we consider the selection coefficients that estimate selection over the duration of the experiment to be the more accurate representation of selection.

For experiments with only a single recapture episode, relative fitness can only have two possible values and selection differentials were calculated by standardizing trait values and then subtracting the mean trait value of survivors from the population mean value. Statistical significance was tested by performing a logistic regression between the standardized trait value and fitness. For these experiments, selection gradients were calculated using multiple logistic regressions. The relevant coefficients resulting from the logistic regressions were converted to their linear equivalents following the methods of Janzen and Stern (1998). For all experiments, linear selection coefficients represent the number of standard deviations that selection will change the mean value of a trait within a generation (Lande and Arnold 1983; Kingsolver et al. 2001).

Given the well-documented challenges associated with detecting significant selection in the wild (Mitchell-Olds and Shaw 1987; Kingsolver et al. 2001; Hersh and Phillips 2004), we set our alpha level at 0.10, but considered *P*-values between 0.05 and 0.10 as less conclusive support for a hypothesis than *P*-values less than 0.05. Furthermore, given the many recent criticisms of Bonferroni corrections (e.g., Nakagawa 2004), we did not perform such corrections when considering the results of individual selection experiments. Instead, we addressed the issue of multiple comparisons by also implementing statistical models that simultaneously considered data from multiple experiments (see below).

To visualize the pattern of natural selection associated with particular color traits in different selection experiments, we generated cubic spline diagrams for each trait in every experiment (Schluter 1988). We do not here present a formal analysis of nonlinear selection because our specific objective was to test hypotheses concerning linear selection and mean trait values (see Introduction). Nonlinear selection will be explored in a future manuscript.

We were also interested in comparing the broader pattern and strength of natural selection within and between predation regimes, combining data from all experiments. For these analyses, differences in temporal interval were approximately standardized by only considering the first recapture episode from each experiment, except in the low-predation experiment M16 in which the longer interval was used (because only four guppies died by the end of the first episode). Also, because we were interested in documenting natural (unmanipulated) selection in the wild, we excluded the Damier River experiments (which were recently

introduced) from these analyses. To compare the pattern of selection between predation regimes, we approximately followed the analysis of covariance (ANCOVA) methods described by Caruso (2000). Using the combined data from the eight experiments, we generated an ANCOVA model that included, as independent variables, standardized traits including body area (due to collinearity we excluded total coloration from this analysis), predation regime, experiment nested within predation regime, and an interaction term between each trait and predation regime. The dependent variable, relative fitness, was calculated separately for each experiment; therefore the coefficients resulting from these ANCOVA models should not be considered formal selection gradients. Statistical significance of the trait-by-predation-regime interaction terms would indicate statistical support for a difference in the pattern and strength of natural selection between predation regimes. We next considered the predation regimes separately and used a similar ANCOVA with independent variables that included traits (excluding total color), experiment, and interaction terms. Models without the interaction terms (none were significant) were then run to estimate regime-wide selection coefficients for each trait. Finally, we generated a model with all experiments pooled and no regime effect; independent variables were experiment and traits (excluding total color). This model estimates universal selection coefficients for each color trait and for body area (there were no significant interactions). To generate comparable selection coefficients for total coloration identical models were constructed with only body area and total coloration as traits.

RELATING SELECTION TO DIVERGENCE

We were interested in qualitatively comparing our estimates of selection to differences in trait values between high- and low-predation regimes. To test for, and characterize, trait differences between predation regimes we used an (analysis of variance) ANOVA to test for an effect of predation regime and experiment (nested within regime) on body area (body size). For each color individually, we used a similar ANOVA model but here included body area as a covariate (i.e., ANCOVA), thus controlling for possible allometric effects of body size. We initially consider models in which the amount of a particular color depended on predation regime, experiment nested within predation regime, body area, and an interaction term between predation regime and body area. No significant interactions between body area and predation regime were found, indicating that we could assume parallel slopes between predation regimes. We then considered a reduced ANCOVA model (separately for each color), wherein a particular color depended on predation regime, experiment nested within predation regime, and body area. From these models, we estimated the least squares adjusted mean values for each color trait associated with each predation regime. Additionally, a discriminant functional analysis (DFA) was employed to identify the axis

of color variation that maximized discrimination between high- and low-predation individuals.

To formally explore the possibility that variation in color trait values among sites is correlated with variation in the strength of linear selection acting on color traits, we calculated the relative areas of the different colors for each experiment (mean area of a color divided by mean body area), and then arcsine square-root transformed these values. Separately for each color trait (black, green, carotenoid color, structural color, and total color), we then used general linear models to determine if selection gradients measured during the experiments were related to these transformed mean trait values. We evaluated two models in this regard, each conducted separately for each color pattern element: (1) to assess the relationship between selection gradients and population-level mean trait values, the first model considered only selection gradients as the predictor variable; and (2) to assess whether such relationships differ among predation regimes the second model also considered predation regime and the interaction between predation regime and selection gradient as the predictor variables.

Results

SAMPLING EFFICIENCY AND MORTALITY RATES

Our estimated recapture efficiencies were high (range = 83%–98%, mean = 90%) (Table 1). The guppies we did not capture were thus assumed to have perished, particularly because we also sampled from pools below and above our study sites. Consistent with our predictions, daily mortality rates were higher, on average, in high-predation experiments (mean = 3.8%, range = 1.2%–7.7%) compared to low-predation experiments (mean = 1.6%, range = 0.6%–2.6%), although statistical support was modest ($P_{1,9} = 0.057$). Similarly, daily killing power was significantly higher on average in our high-predation experiments (mean = 0.013, range = 0.011–0.019), compared to low-predation experiments (mean = 0.0074, range = 0.003–0.012) ($P_{1,9} = 0.012$; Table 1). Note, however, that some estimates of mortality rate and killing power in low-predation experiments were higher than some estimates in high-predation experiments (Table 1). Overall, mortality rates for our high- (HP) and low-predation (LP) experiments were in the same ranges as those reported for mature males in previous work: Rodd and Reznick 1991 (LP = 3.8%), Reznick et al. 1996 (HP average = 3.8%, LP average = 2.0%) (estimated from Fig. 2C), Olendorf et al. 2006 (HP = 1.6%–2.2%, LP = 1.3%–2.5%), Van Oosterhout et al. 2007 (LP = 1.2%), and Gordon et al. 2009 (HP = 0%–5.0%, LP = 0.5%–2.0%).

ESTIMATES OF NATURAL SELECTION

We first consider the results of individual selection experiments. Results for selection differentials and gradients were similar, and

Table 2. Linear selection gradients (β) associated with all traits in the 10 selection experiments.

Experiment	Size		Black		Green		Carot		Struct		Total	
	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>	β	<i>P</i>
Low predation												
M16	-0.05	0.54	-0.02	0.72	-0.05	0.47	0.02	0.66	-0.02	0.81	-0.01	0.84
M1	0.15	0.22	-0.17	0.07	-0.07	0.51	-0.12	0.18	-0.23	0.06	-0.30	0.01
M10	-0.01	0.89	-0.01	0.89	-0.02	0.70	-0.11	0.06	-0.06	0.39	-0.16	0.001
AL	-0.08	0.75	0.01	0.94	-0.08	0.58	-0.26	0.09	-0.32	0.10	-0.31	0.11
DL	0.01	0.92	-0.03	0.64	-0.08	0.20	0.03	0.71	-0.04	0.55	-0.05	0.49
High predation												
M15	-0.01	0.93	-0.16	0.08	-0.07	0.41	-0.13	0.14	0.00	0.99	-0.24	0.01
M17	0.10	0.61	-0.10	0.55	-0.16	0.33	-0.11	0.52	0.07	0.71	-0.15	0.39
AH05	-0.11	0.71	0.32	0.10	-0.03	0.89	-0.06	0.74	-0.13	0.54	0.19	0.41
AH06	0.06	0.67	-0.14	0.19	0.01	0.90	0.08	0.45	-0.13	0.26	-0.11	0.40
DH	0.02	0.86	0.09	0.39	0.15	0.21	-0.12	0.22	-0.02	0.87	0.02	0.87

so we here discuss results for gradients only, with results for differentials appearing in the Supporting Information (Table S2). Consistent with expectations, the majority of estimated selection gradients (for individual experiments considered separately) for color were negative (Table 2, Figs. 1 and 2). Of the seven gradients that were significant ($P < 0.10$), six were negative. The only significant case of positive selection on color was for black coloration in one selection experiment (2005) at the Aripo high-predation site (Table 2, Fig. 1). The absolute values of significant selection gradients measured in this study range from 0.11 to 0.32, which exceed 40%–78% (respectively) of the gradients from the literature (Kingsolver et al. 2001). We did not detect significant selection gradients associated with fish body size (body area).

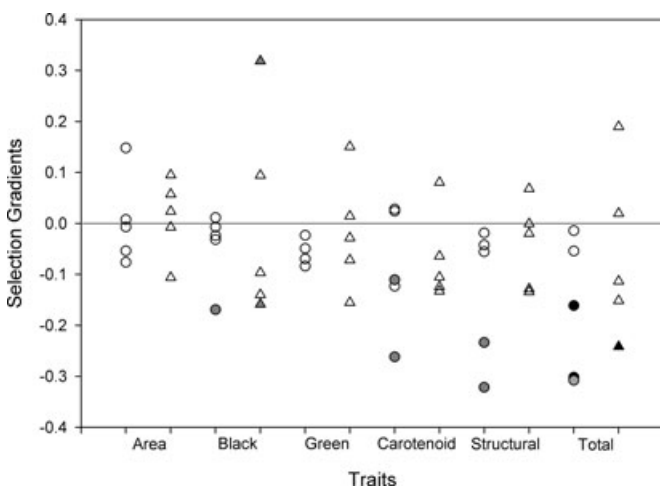


Figure 1. Selection gradients for all experiment/trait combinations considered in this study. Circles represent gradients associated with low-predation experiments. Triangles represent gradients associated with high-predation experiments. Statistical support is represented by the shading in the symbols interiors (gray fill $P < 0.1$; black fill $P < 0.05$).

Significant selection gradients were most commonly associated with black (i.e., the aforementioned estimate from Aripo high predation), carotenoid colors, structural colors, and total color (Table 2, Fig. 1). Green appeared to be the most selectively benign color: no selection gradients associated with green were significant (Table 2, Fig. 1). Power to detect significant selection coefficients can be influenced by sample size (Kingsolver et al. 2001; Hersh and Phillips 2004). However, we sometimes failed to detect statistically significant selection in experiments with relatively large sample sizes (e.g., M17 = 111, AH06 = 210) and, in other cases, detected selection with comparatively modest sample sizes (e.g., AH05 = 100, AL = 95). These results suggest that sample size did not strongly influence the patterns of selection that we detected.

Differences in the prevalence and strength of selection between high- and low-predation experiments were not as overt as we had expected (Figs. 1 and 2). Indeed, more bouts of significant selection against color components or total color were encountered in low-predation experiments than in high-predation experiments (Table 2, Fig. 1). Our ANCOVA analyses, combining all selection experiments, revealed no support for differences in selection associated with any color traits between high-predation and low-predation experiments (interaction terms: Table 3), suggesting that the magnitude and direction of selection was similar in both regimes. Therefore, we focus on interpreting the results of the ANCOVA models that estimated universal selection gradients (experiments from both regimes pooled). In these models, selection against structural and total color was strong and well supported statistically, but coefficients associated with body area, green color, black color, and carotenoid color were not.

Fluctuating selection was occasionally suggested by comparisons of selection gradients (Table 4) and differentials (Table S3) between the early and late episodes for the five experiments with

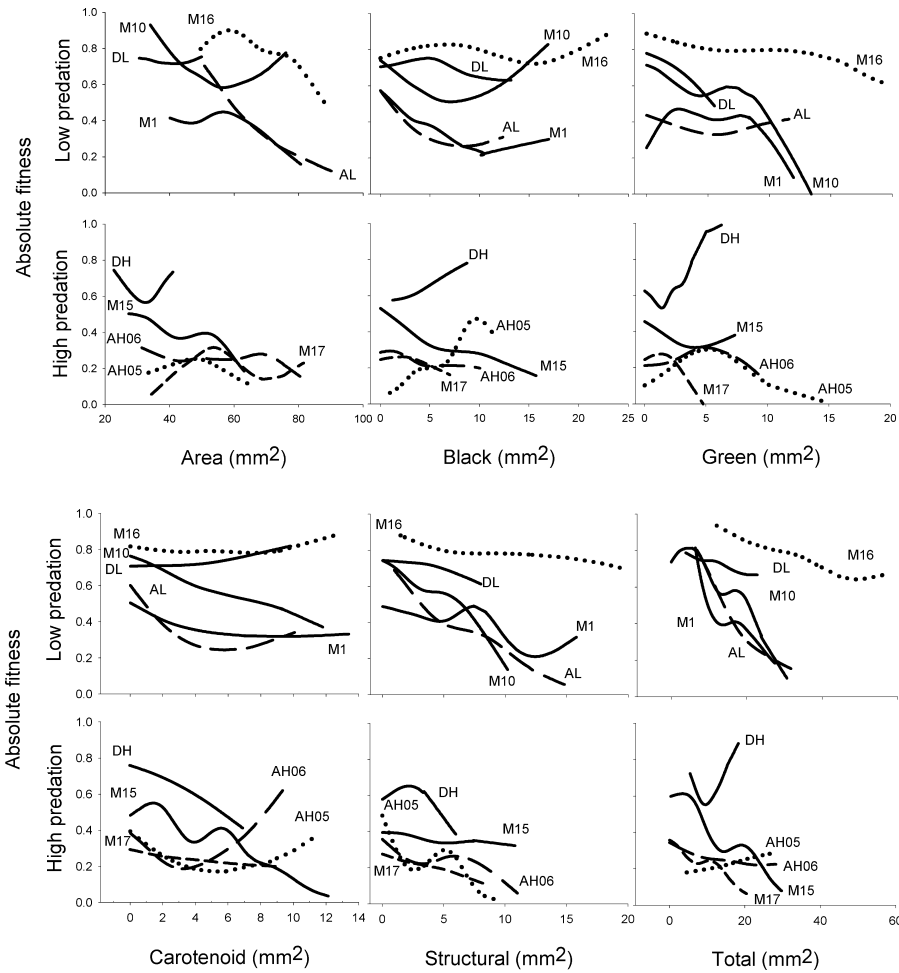


Figure 2. Cubic splines depicting the relationship between various traits considered in this study and absolute fitness. To facilitate interpretation, experiments with lines that are very close together (on the figure) have been assigned different dash patterns.

two sequential recapture events (M1, M10, M16, M17, AH06). For example, in the high-predation experiment at AH06 the sign of selection gradients for four of five color traits were reversed in the second recapture episode. In this experiment, selection gradients for green, carotenoid, structural, and total colors were negative in the first episode (only structural and total were significant), and positive in the second episode (all significant except total color).

Note that for this experiment, the selection gradients that estimated selection over the entire duration of the experiment (both recapture episodes) were insignificant for all traits (Table 2).

RELATING SELECTION TO DIVERGENCE

Consistent with previous work, male guppies from low-predation experiments were larger, on average, than were those from

Table 3. Summary of results of the ANCOVA models that (1) tested for significant differences in selection between the two putative regimes (Trait×Regime *P*), (2) estimated selection coefficients separately within low-predation (LP β) and high-predation regimes (HP β), and (3) estimated universal selection coefficients (Universal β) with experiments from both regimes pooled. These analyses do not include the Damier River experimental introductions.

Trait	Trait×Regime <i>P</i>	LP β	<i>P</i>	HP β	<i>P</i>	Universal β	<i>P</i>
Area	0.91	-0.024	0.70	-0.01	0.92	-0.027	0.65
Black	0.82	-0.031	0.41	-0.013	0.86	-0.23	0.57
Green	0.65	-0.027	0.50	-0.068	0.40	-0.033	0.45
Carotenoid	0.17	-0.048	0.18	-0.152	0.02	-0.094	0.128
Structural	0.33	-0.097	0.028	-0.20	0.037	-0.12	0.011
Total	0.28	-0.13	0.0078	-0.24	0.011	-0.17	0.001

Table 4. Selection gradients calculated separately for early (β_1) and late (β_2) recapture episodes (* $P < 0.1$, ** $P < 0.05$; only experiments with two recapture episodes).

Experiment	Area		Black		Green		Carotenoid		Structural		Total	
	β_1	β_2	β_1	β_2	β_1	β_2	β_1	β_2	β_1	β_2	β_1	β_2
M1	0.15	0.00	-0.05	-0.23	-0.06	-0.02	-0.05	-0.15	-0.17*	-0.13	-0.15	-0.33**
M10	-0.03	0.04	0.03	-0.07	0.02	-0.09	-0.07	-0.07	-0.07	0.03	-0.09**	-0.16**
M17	-0.04	0.23	-0.09	-0.02	-0.07	-0.08	-0.18	0.13	0.06	-0.04	-0.19	0.03
M16	0.12	-0.13	0.06	-0.07	-0.04	-0.08	0.05	0.02	-0.04	-0.02	0.04	-0.07
AH06	0.02	0.12	-0.02	-0.33*	-0.08	0.31**	-0.03	0.26*	-0.24**	0.28*	-0.17	0.16

high-predation experiments (Table S4). Also, our DFA identified body size as an important variable discriminating between predation regimes (Table S4, Fig. S1). We also found significant differences in coloration between males from high- and low-predation experiments. Consistent with previous work, low-predation guppies were more colorful for their size than were high-predation guppies (Table S4, Fig. S1), particularly in structural colors. Note, again, however, that for each color trait average values overlapped somewhat between high- and low-predation experiments: for example, some high-predation experiments had more structural color than some low-predation experiments. Moreover, not every color category followed the predictions; high-predation guppies actually had a greater total area of carotenoid color spots for their size than did low-predation guppies (Table S4, Fig. S1). The multivariate DFA supported this trend, with low-predation guppies toward the structural color end of the discriminant function and high-predation guppies toward the carotenoid color end (Table S4, Fig. S1). Low-predation populations had more black and green, but these colors did not load as strongly on the DFA (Table S1, Fig. S1).

Analyses of the relationship between experiment-specific values of male color and experiment-specific estimates of selection were significant for only a single color trait. Here we detected a significant negative relationship between the strength of selection against structural colors and the mean relative area of structural colors ($R^2 = 0.46$, $F_{1,9} = 6.71$, $P = 0.032$, Fig. 3). Experiments with initially more structural color experienced stronger selection against structural coloration. We found no evidence that the relationship between selection and trait values differed between predation regimes (all interactions were insignificant).

Discussion

Our objective was to obtain the first formal linear estimates of selection on male guppy coloration from replicated mark-recapture experiments in the wild, and to then use those replicate estimates to test a priori hypotheses about the role of nature selection in shaping geographic patterns of male color variation. Our results broadly support many existing perspectives about the se-

lective basis for color variation, but also suggest some important nuances.

PREVALENCE AND STRENGTH OF NATURAL SELECTION

Consistent with our first hypothesis, the presumed effects of predators, and several other guppy mark-recapture studies (Reznick et al. 1996; Gordon et al. 2009), mortality rates were, on average, higher in high-predation experiments than in low-predation experiments. However, mortality rates were also quite variable, especially in high-predation experiments (Table 1). It is worth noting that not all of our unrecaptured guppies were necessarily eaten by aquatic predators; other agents of mortality

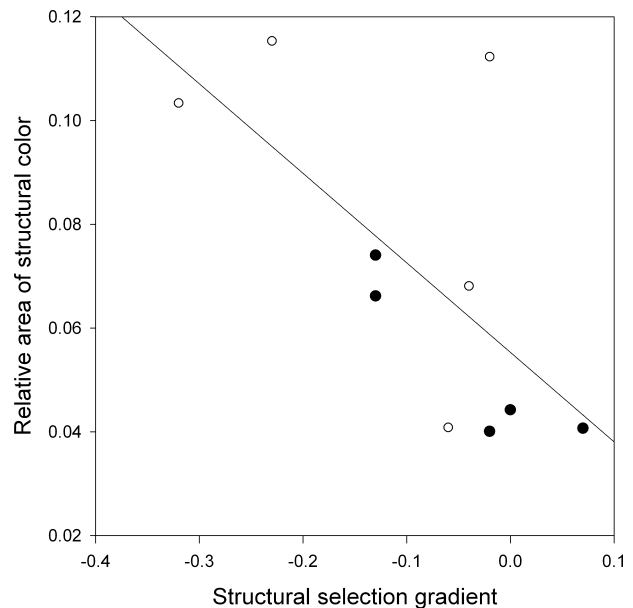


Figure 3. Relationship between the strength of selection on structural color and the average amount of structural color among the 10 experiments ($R^2 = 0.46$, $F_{1,9} = 6.71$, $P = 0.032$). Open symbols represent low-predation experiments. Filled symbols represent high-predation experiments. The negative relationship suggests that we measured stronger selection against structural coloration in experiments that had higher initial mean values for structural color.

(starvation, parasitic infection, or bird predators) may account for some of the variable mortality in our mark–recapture experiments. It would be very useful to consider these factors in future work.

Our second hypothesis was that selective trade-offs between natural and sexual selection in guppies should cause more colorful male guppies to experience greater mortality. Consistent with this prediction, selection coefficients for color pattern elements were mostly negative, directly confirming for the first time that viability selection in the wild generally disfavors male guppies with exaggerated color patterns. Within this general pattern, however, selection varied extensively in space and time. Indeed, in one high-predation experiment in particular (AH06), separate selection gradients for the early and late recapture episodes (of the same selection experiment) revealed opposite directions of selection—a complexity that was obscured by selection estimates that spanned both recapture intervals. Without experimental manipulation of potential causative agents, we can only speculate about specific causes of this spatiotemporal variation in selection. One possibility is frequency-dependent selection by predators results in a rare-type viability advantage (Olendorf et al. 2006). Another possibility is spatiotemporal variation in the abundance and distribution of predators or other environmental factors that influence risk of predation, such as light availability and spectral properties of the water, both of which could vary spatially and temporally (e.g., increased turbidity during periods of higher rainfall).

Spatiotemporal variation in selection is not entirely unexpected (Siepielski et al. 2009). For example, general reviews of selection and contemporary evolution in the wild (Hendry and Kinnison 1999; Hoekstra et al. 2001; Kinnison and Hendry 2001) indicate that estimates over longer periods of time (multigenerational) are weaker, probably owing to a tendency for temporal averaging over periods of fluctuating selection across generations. Even within generations or cohorts, however, conflicting selection on a trait during an individual's ontogeny is predicted to result in evolutionary trade-offs that constrain the direction of evolution (Schluter et al. 1991). Indeed, consistent directional selection might be relatively rare outside of specific contexts; for example, immediately following the colonization of a novel habitat (Clegg et al. 2008), in the face of ongoing gene flow (Bolnick and Nosil 2007), or owing to human perturbations (Darimont et al. 2009). That said, our own assessment of selection in introduction sites on the Damier River (9 years after these population were established) did not reveal any significant selection.

The biological relevance of spatiotemporal variation in selection has been stressed in recent work examining the spatial scale of adaptation (Svensson and Sinervo 2004; Garant et al. 2007), apparent trait stasis (Hendry and Kinnison 1999; Merilä et al. 2001), the maintenance of genetic variation (Roff 1997), and the evolution of phenotypic plasticity (Huber et al. 2004). Perhaps

one of the most enduring questions in guppy biology surrounds hypothesized mechanisms that maintain extreme levels of male color polymorphisms. Thus far, support has been provided for the roles of mate choice (Endler and Houde 1995; Hughes et al. 1999; Gamble et al. 2003), frequency-dependent selection (Olendorf et al. 2006), and negative genetic correlation between attractiveness and survival (Brooks 2000). Here we provide evidence that high spatiotemporal variation in natural selection, coupled with the relatively short life span of guppies, should be considered an additional candidate mechanism (but see Hedrick 2000 for a theoretical discussion of the role of spatiotemporal variation in maintaining genetic polymorphism).

The pattern and strength of spatiotemporal variation in selection also have important consequences for a broader set of eco-evolutionary interactions (Yoshida et al. 2003; Hanski and Saccheri 2006; Kinnison and Hairston Jr. 2007; Pelletier et al. 2007). Clearly, any broader understanding of the ecological relevance of ongoing evolutionary processes requires not only a demonstration of the population, community, and ecosystem consequences of intraspecific trait variation (e.g., Palkovacs et al. 2009), but also studies that reveal the spatial and temporal grain over which selection and evolution may shape such dynamics (Siepielski et al. 2009). The variation in selection documented in this study suggests that such eco-evolutionary effects might occur on very fine spatiotemporal scales.

REGIME-SPECIFIC SELECTION

Given the general pattern of selection against color and the considerable variability in selection among mark–recapture experiments, it is noteworthy that we did not find support for the hypothesis that the strength of selection is generally greater in high-predation habitats (hypothesis 3). This can be seen by considering patterns in the experiment-specific selection estimates and in our ANCOVA analyses that combined experiments. Despite evidence of higher mortality rates in high-predation experiments, experiment-specific estimates detected significant selection against color in only a single high-predation experiment (M15). In contrast, selection against color was evident for at least three color traits in three of five low-predation experiments (Fig. 1; Table 2). When we combined our multiple experiments into a single ANCOVA analysis comparing predation regimes, we found support for universal selection against structural color and total color, but no significant regime-by-color interaction terms that would suggest differences in selection between the high- and low-predation habitats at large. On the other hand, this finding is consistent with suggestions by some authors that predators in low-predation sites (e.g., *Rivulus harti* and *Macrobrychium* spp.) might impose significant mortality and color selection in their own right (Endler 1978, 1980, 1983; Millar et al. 2006; Kemp et al. 2008; McKellar et al. 2009).

The fact that we did not find evidence of divergent selection between predation regimes warrants further discussion. It is difficult to rule out the possibility that our spatiotemporal replication of selection experiments (which estimated selection during relatively narrow windows of time) was inadequate to detect some rare, but strong, bouts of natural selection that might have disproportionate effects on color traits in high-predation sites. Some previous authors have also suggested that predators are not the only environmental factor shaping geographic variation in male color in Trinidadian guppies. Other environmental variables that may mediate color pattern evolution include canopy openness, primary production, and variation in water transmission properties (Kodric-Brown 1989; Grether et al. 1999, 2001; Millar et al. 2006). Guppy populations are known to differ in susceptibility to parasite infection (Van Oosterhout et al. 2003), such variation may also influence color pattern evolution through viability and sexual selection (Houde and Torio 1992; Van Oosterhout et al. 2003).

RELATING SELECTION TO DIVERGENCE

Although we did not detect significant differences in selection at a regime level, it is important to note that we did nonetheless detect differences in male color between predation regimes. Phenotypic color comparisons from this study were broadly consistent with the major conclusions of previous studies comparing males from high- and low-predation sites (Endler 1978, 1983; Reznick 1982; Reznick and Endler 1982; Magurran 2005; Millar et al. 2006; Kemp et al. 2008). For example, our observation of greater structural coloration (i.e., blues and iridescent colors) in low-predation experiments (compared to high-predation experiments) is similar to earlier surveys and experiments (Endler 1978, 1980). In contrast, we did not find greater carotenoid color in low-predation experiments, but other studies have also found variable results in this regard (Endler 1978, 1980, 1983). Likewise, recent studies that have compared the coloration of high- and low-predation guppies have documented a diverse range of outcomes despite some generally recognized trends (e.g., Alexander et al. 2006; Millar et al. 2006; Karim et al. 2007; Schwartz and Hendry 2007; Kemp et al. 2008).

Several explanations have been advanced for why variable results are obtained when comparing high- and low-predation guppy populations (Millar et al. 2006; Schwartz and Hendry 2007; Kemp et al. 2008). We can here address one of these explanations: divergent selection might be temporally variable or even episodic. Under these conditions, different geographic surveys of standing variation might yield different patterns, particularly if strong bouts of selection had recently acted in different populations in different studies. In this framework, focused and temporally replicated studies of selection in the wild can supplement surveys of geo-

graphical variation in trait values (e.g., Endler 1978; Alexander et al. 2006; Millar et al. 2006) by drawing attention to the finer dynamics that likely contribute to, but also complicate, geographic patterns.

Irrespective of regime-specific patterns of selection and color, it is nonetheless useful to inquire whether experiment-specific patterns of selection are in any way related to local color variation (hypothesis 4). In general, patterns of contemporary viability selection did not strongly predict broad patterns of trait divergence. If so, we would have expected stronger selection against color to be associated with less male coloration. If anything, the converse appears to be truer—greater amounts of structural color were associated with stronger selection against structural colors (e.g., Fig. 3). This might suggest that the detection of selection on structural color is in part linked to the phenotypic opportunity for selection (i.e., the total amount of color variation present). For most color traits, however, no clear associations were detected, which may suggest that past selection and local adaptation have reduced the phenotypic opportunity for contemporary selection on some traits (e.g., Clegg et al. 2008), particularly in high-predation sites.

The discordant relationship between patterns of natural selection and trait variation (greater areas of particular colors in experiments in which those colors are disfavored by natural selection) might best be explained by dynamic trade-offs between natural (i.e., viability) selection and sexual selection. In some sites, stronger sexual selection for male color might pull male traits further from the optimal with respect to natural selection, and thereby generate stronger, and detectable, natural selection. Of course, this hypothesis in turn raises the question of what factors might cause spatiotemporal variation in the strength or pattern of sexual selection?

Research on sexual conflict has revealed that male guppies can obtain fertilizations either by attracting a female through mating displays or through coercive mating behaviors (Houde 1997; Godin 1995; Magurran 1998, 2005). The elevated mortality risk in high-predation sites might favor males that engage in sneaky copulation attempts, instead of complex mating displays (Godin 1995). Moreover, guppies in high-predation sites often show more schooling behavior (Seghers and Magurran 1995), which might further enhance opportunities for males to succeed in coercive matings. Finally, females might be less attracted to, or discriminating against, displaying males if such displays increase predation risks for females (Godin and Briggs 1996). Accordingly, some studies have reported that female preferences for male colors are weaker in high-predation sites (Schwartz and Hendry 2007). Collectively, these possibilities suggest that predator environment could influence net selection on color indirectly through its effects on the strength and pattern of sexual selection.

In short, geographical patterns of color variation may in some cases be more directly explained by environmental (e.g., predatory) modulation of sexual selection than by the direct effects of viability selection. However, additional studies that simultaneously estimate natural and sexual selection (e.g., Hamon and Foote 2005 and Sennsson et al. 2006) would be required to formally address this hypothesis in the guppy system. Interestingly, sexual selection gradients have been estimated for low-predation guppies under laboratory conditions by Brooks and Endler (2001). In that study, estimates of sexual selection gradients for areas of black, iridescent and orange colors were comparable in scale but opposite in sign to our estimates of natural selection (0.077, 0.205 and 0.127 respectively: Brooks and Endler 2001). Such comparability of scale, but opposing sign, suggests that natural and sexual selection might interact strongly, leading to diverse and unstable evolutionary outcomes for male color at various sites and times.

SUMMARY

We documented considerable spatiotemporal variation in viability selection both within and among the classically categorized predation regimes experienced by wild guppy populations. Our study supports previous inferences in that mortality rates are greater in high-predation sites, and that natural selection broadly disfavors male guppies with more color. However, it does not support the prediction that natural selection against color would generally be stronger in high-predation sites. Indeed, natural selection measured in any given low-predation experiment was often as strong, or stronger, than natural selection measured in any given high-predation experiment. Moreover, patterns of natural selection did not readily predict geographic variation in guppy color. We suggest that one explanation for this discordance with geographical patterns of color divergence may be that the role of visual-hunting fish predators should be deconstructed into direct effects (viability selection by predators against colorful males) and indirect effects resulting from predation's influence on sexual behavior (e.g., decreased sexual selection favoring colorful males in habitats with higher extrinsic mortality). Real-time studies of the interaction of natural and sexual selection in the wild, perhaps using natural pedigrees, could ultimately yield more definitive insights into the relative importance of these components of selection on male color in this classic evolutionary system.

ACKNOWLEDGMENTS

This study has benefited from discussions with and comments provided by M. Bailey, W. Glanz, I. Kornfield, D. Reznick, J. Walker, E. Svensson, and an anonymous reviewer. P. Bentzen, M. Bailey, C. Blackie, L. Easty, C. Holbrook, M. Kelly, N. Millar, K. Lachapelle, C. Lage, B. Libby, I. Patterson, A. Schwartz, and N. Wilke provided valuable assistance in the field. J. DiBattista gave helpful guidance on selection analyses. This research was funded by the National Science Foundation (DEB 0235605) and is contribution number 3098 of the Maine Agricultural and Forest Experiment Station.

LITERATURE CITED

- Alexander, H. J., J. S. Taylor, S. S. Wu, and F. Breden. 2006. Parallel evolution and vicariance in the guppy (*Poecilia reticulata*) over multiple spatial and temporal scales. *Evolution* 60:2352–2369.
- Barton, N. H., and M. Turelli. 1989. Evolutionary quantitative genetics: how little do we know? *Annu. Rev. Genet.* 23:337–370.
- Begon, M., M. Mortimer, and D. J. Thompson. 1996. *Population Ecology: a unified study of animals and plants*. Blackwell Science, Oxford, U.K.
- Bolnick, D. I., and P. Nosil. 2007. Natural selection in populations subject to a migration load. *Evolution* 61:2229–2243.
- Brodie, E. D., and F. J. Janzen. 1996. On the assignment of fitness values in statistical analyses of selection. *Evolution* 50:437–442.
- Brodie, E. D., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends Ecol. Evol.* 10:313–318.
- Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature* 406:67–70.
- . 2002. Variation in female mate choice within guppy populations: population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica* 116:343–358.
- Brooks, R., and J. A. Endler. 2001. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* 55:1002–1015.
- Bryant, M. J., and D. Reznick. 2004. Comparative studies of senescence in natural populations of guppies. *Am. Nat.* 163:55–68.
- Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* 54:1546–1557.
- Clegg, S. M., F. D. Frentiu, J. Kikkawa, G. Tavecchia, and I. P. F. Owens. 2008. 4000 years of phenotypic change in an island bird: heterogeneity of selection over three microevolutionary timescales. *Evolution* 62:2393–2410.
- Crispo, E., P. Bentzen, D. N. Reznick, M. T. Kinnison, and A. P. Hendry. 2006. The relative influence of natural selection and geography on gene flow in guppies. *Mol. Ecol.* 15:49–62.
- Croft, D. P., B. Albanese, B. J. Arrowsmith, M. Botham, M. Webster, and J. Krause. 2003. Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia* 137:62–68.
- Darimont, C. T., S. M. Carlson, M. R. Kinnison, P. C. Paquet, T. E. Reimchen, and C. C. Wilmers. 2009. Human predators outpace other agents of trait change in the wild. *Proc. Natl. Acad. Sci. USA* 106:952–954.
- Endler, J. A. 1978. A predator's view of animal color patterns. *Evol. Biol.* 11:319–364.
- . 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* 34:76–91.
- . 1983. Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fish.* 9:173–190.
- . 1986. *Natural selection in the wild*. Princeton Univ. Press, Princeton, NJ.
- . 1995. Multiple trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* 10:22–29.
- Endler, J. A., and A. E. Houde. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* 49:456–468.
- Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford Univ. Press, Oxford, U.K.
- Gamble, S., A. K. Lindholm, J. A. Endler, and R. Brooks. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behavior and sexual selection in guppies. *Ecol. Lett.* 6:463–472.
- Garant, D., L. E. B. Kruuk, R. H. McCleery, and B. C. Sheldon. 2007. The effects of environmental heterogeneity on multivariate selection on reproductive traits in female great tits. *Evolution* 61:1546–1559.

- Godin, J.-G. J. 1995. Predation risk and alternative mating tactics in male Trinidadian guppies. *Oecologia* 103:224–229.
- Godin, J. G. J., and S. E. Briggs. 1996. Female mate choice under predation risk in the guppy. *Anim. Behav.* 51:117–130.
- Godin, J. J., and H. E. McDonough. 2003. Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behav. Ecol.* 14:194–200.
- Gordon, S. P., D. N. Reznick, M. T. Kinnison, M. J. Bryant, D. J. Weese, K. Räsänen, N. P. Millar, and A. P. Hendry. 2009. Adaptive changes in life history and survival following a new guppy introduction. *Am. Nat.* 174:34–45.
- Grether, G. F., J. Hudon, and D. F. Millie. 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc. R. Soc. Lond. B* 266:1317–1322.
- Grether, G. F., J. Hudon, and J. E. Endler. 2001. Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies (*Poecilia reticulata*). *Proc. R. Soc. Lond. B* 268:1245–1253.
- Hamon, T. R., and C. J. Foote. 2005. Concurrent natural and sexual selection in wild male sockeye salmon, *Oncorhynchus nerka*. *Evolution* 59:1104–1118.
- Hanski, I., and I. Saccheri. 2006. Molecular-level variation affects population growth in a butterfly metapopulation. *Plos Biol.* 4:719–726.
- Hedrick, P. W. 2000. *Genetics of populations*. Jones and Bartlett Publishers, Sudbury, MA.
- Hendry, A. P., and M. T. Kinnison. 1999. The pace of modern life: measuring rates of contemporary microevolution. *Evolution* 53:1637–1653.
- Hersh, E. I., and P. C. Phillips. 2004. Power and potential bias in field studies of natural selection. *Evolution* 58:479–485.
- Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. *Proc. Natl. Acad. Sci. USA* 98:9157–9160.
- Houde, A. E. 1987. Mate choice based upon naturally occurring colour-pattern variation in a guppy population. *Evolution* 41:1–10.
- . 1997. *Sexual selection and mate choice in guppies*. Princeton Univ. Press, Princeton, NJ.
- Houde, A. E., and A. J. Torio. 1992. Effects of parasite infection on male colour pattern and female choice in guppies. *Behav. Ecol.* 3:346–351.
- Huber, H., N. C. Kane, M. S. Heschel, E. J. von Wettberg, J. Banta, A. Leuck, and J. Schmitt. 2004. Frequency and microenvironmental pattern of selection on plastic shade-avoidance traits in a natural population of *Impatiens capensis*. *Am. Nat.* 163:548–563.
- Hughes, K. A., L. Du, F. H. Rodd, and D. N. Reznick. 1999. Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*. *Anim. Behav.* 58:907–916.
- Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* 52:1564–1571.
- Karim, N., S. P. Gordon, A. K. Schwartz, and A. P. Hendry. 2007. This is not déjà vu all over again: male guppy colour in a new experimental introduction. *J. Evol. Biol.* 20:1339–1350.
- Kemp, D. J., D. N. Reznick, and G. F. Grether. 2008. Ornamental evolution in Trinidadian guppies (*Poecilia reticulata*): insights from sensory processing-based analyses of entire colour patterns. *Biol. J. Linn. Soc.* 95:734–747.
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, N. Vignieri, C. E. Hill, A. H. Hoang, P. Gilbert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. *Am. Nat.* 157:245–261.
- Kinnison, M. T., and N. G. Hairston. 2007. Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. *Funct. Ecol.* 21:444–454.
- Kinnison, M. T., and A. P. Hendry. 2001. The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* 112–113:145–164.
- 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.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Magurran, A. E. 1998. Population differentiation without speciation. *Philos. Trans. R. Soc. Lond. B* 353:275–286.
- . 2005. *Evolutionary ecology: the Trinidadian guppy*. Oxford Univ. Press, Oxford, U.K.
- Merilä, J., B. C. Sheldon, and L. E. B. Kruuk. 2001. Explaining stasis: microevolutionary studies in natural populations. *Genetica* 112–113:119–222.
- McKellar, A. E., M. M. Turcotte, and A. P. Hendry. 2009. Environmental factors influencing adult sex ratio in Trinidadian guppies. *Oecologia* 159:735–745.
- Millar, N. M., D. N. Reznick, M. T. Kinnison, and A. P. Hendry. 2006. Disentangling the selective factors that act on male coloration in wild guppies. *Oikos* 113:1–12.
- Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* 41:1149–1161.
- Nakagawa, S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav. Ecol.* 15:10044–1045.
- Olendorf, R., F. H. Rodd, D. Punzalan, A. E. Houde, C. Hurt, D. N. Reznick, and K. A. Hughes. 2006. Frequency-dependent survival in natural guppy populations. *Nature* 441:633–636.
- Palkovacs, E. P., M. C. Marshall, B. A. Lamphere, B. R. Lynch, D. J. Weese, D. F. Fraser, D. N. Reznick, C. M. Pringle, and M. T. Kinnison. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. Trans. R. Soc. Lond. B* 364:1617–1628.
- Pelletier, F., T. Clutton-Brock, J. Pemberton, S. Tuljapurkar, and T. Coulson. 2007. The evolutionary demography of ecological change: linking trait variation and population growth. *Science* 315:1571–1574.
- Reznick, D. N. 1982. The impact of predation on life history evolution in Trinidadian guppies: the genetic components of observed life history differences. *Evolution* 36:1285–1297.
- Reznick, D., and J. A. Endler. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36:160–177.
- Reznick, D. N., M. J. Butler, F. H. Rodd, and P. Ross. 1996. Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution* 50:1651–1660.
- Rodd, F. H., and D. N. Reznick. 1991. Life History Evolution in Guppies: III. The impact of prawn predation on guppy life histories. *Oikos* 62:13–19.
- Roff, D. A. 1997. *Evolutionary quantitative genetics*. Chapman and Hall, International Thompson Publishing, Toronto, Canada.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42:849–861.
- . 2000. *The ecology of adaptive radiation*. Oxford Univ. Press, Oxford, U.K.
- Schluter, D., T. D. Price, and L. Rowe. 1991. Conflicting selection pressures and life-history trade-offs. *Philos. Trans. R. Soc. Lond. B* 246:11–17.
- Schwartz, A. K., and A. P. Hendry. 2007. A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*). *Evol. Ecol. Res.* 9:71–90.

- Seghers, B. H., and A. E. Magurran. 1995. Population differences in the schooling behavior of the Trinidadian guppy, *Poecilia reticulata*—adaptation or constraint. *Can. J. Zool.* 73:1100–1105.
- Siepielski, A. M., J. D. DiBattista, and S. M. Carlson. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecol. Lett.* 12:1261–1276.
- Svensson, E. I., and B. Sinervo. 2004. Spatial scale and temporal component of selection in side-blotched lizards. *Am. Nat.* 163:726–734.
- Svensson, E. I., and T. P. Gosden. 2007. Contemporary evolution of secondary sexual traits in the wild. *Funct. Ecol.* 21:422–433.
- Svensson, E. I., F. Eroukmanoff, and M. Friberg. 2006. Effects of natural and sexual selection on adaptive population divergence and premating isolation in a damselfly. *Evolution* 60:1242–1253.
- Van Oosterhout, C., P. D. Harris, and J. Cable. 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., R. S. Mohammed, H. Hansen, G. A. Archard, M. McMullan, D. J. Weese, and J. Cable. 2007. Selection by parasites in space conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int. J. Parasitol.* 37:805–812.
- Yoshida, T., L. E. Jones, S. P. Ellner, G. F. Fussman, and N. G. Hairston, Jr. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424:303–306.

Associate Editor: M. Webster

Supporting Information

The following supporting information is available for this article:

Figure S1. Visual summary of discriminant function analysis (DFA) that differentiated between high- and low-predation sites based on body area and color traits (black, green, carotenoid, and structural).

Table S1. Locations of the sites used for our selection experiments in Trinidad's Northern range mountains.

Table S2. Linear selection differentials (S) associated with all traits in the 10 selection experiments.

Table S3. Selection differentials calculated separately for early ($S1$) and late ($S2$) recapture episodes ($*P < 0.1$, $**P < 0.05$) (only experiments with two recapture episodes).

Table S4. Population trait means in millimeter square (\pm SEM) for all 10 sites.

Supporting Information may be found in the online version of this article.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.