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Testing the influence of local forest canopy clearing on phenotypic variation in Trinidadian guppies

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Summary

1. Factors contributing to the maintenance of phenotypic variation in nature are often difficult to determine. Secondary sexual traits might be particularly interesting in this regard due to the interaction they experience between multiple selective agents. One way to examine such effects is to monitor populations following environmental change. Human-caused changes can be particularly useful here because they often involve an abrupt and extreme alteration of specific habitat features. This alteration can then precipitate phenotypic plasticity, changes in adaptive landscapes, and modified evolutionary trajectories. The consequences of habitat manipulations on local populations can therefore improve our understanding of phenotypic variation in complex ecological systems.

2. We took advantage of a human-caused environmental disturbance to examine factors influencing phenotypic variation in Trinidadian guppies (*Poecilia reticulata*). Differences in canopy cover along the stream have been hypothesized to explain some of this variation, but this has been hard to test directly. We here attempt a direct test of this hypothesis by monitoring changes in guppy size and colour following a dramatic decrease in canopy cover due to tree removal for agricultural activity.

3. Although male and female body size increased following canopy clearing, little change was observed in the overall amount of melanin-based colours, carotenoid-based colours, and structural colours on males. We further compared phenotypes before and after canopy clearing at the disturbed site to those from two nearby reference sites that are at extreme ends of canopy cover. Overall, variation in colour was attributed to differences among sites, irrespective of canopy differences. We also found considerable temporal variation in some colour elements at a given site.

4. Our results suggest that differences in light availability do not cause rapid and dramatic changes in guppy colour. The substantial unexplained variation must therefore be due to factors other than canopy (measured here) and predation regime (all sites were 'low-predation'). Because of the multiple and complex interactions involved in the expression and maintenance of sexually selected traits, our study emphasizes the need for a better understanding of both the genetic and environmental sources of co-variation between sexual ornaments and preferences.

Key-words: canopy cover, disturbance, microhabitat selection, phenotypic change, *Poecilia reticulata*, sexual selection

Introduction

The pace of adaptation in natural populations is an important question from an academic perspective (Hendry & Kinnison 1999; Kinnison & Hendry 2001; Estes & Arnold 2007; Fisk *et al*. 2007). More recently, its applied relevance has become increasingly apparent (Burger & Lynch 1995; Stockwell, Hendry & Kinnison 2003; Bell & Collins 2008). One common question is whether organisms are capable of responding adaptively so as to avoid population declines in the face of environmental change (Gomulkiewicz & Holt 1995; Willi, Buskirk & Hoffman 2006; Kinnison & Hairston 2007; Visser 2008). These concerns can be particularly relevant in the case of human-caused disturbances, because these often surpass the natural baseline of environmental perturbations (Vitousek *et al*. 1997; Palumbi 2001).

If we are to understand the effects of human disturbance on evolutionary and ecological processes, we must first understand the main environmental drivers in a given situation. Although adaptive phenotypic changes have now been

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*Correspondence author. E-mail: amy.schwartz@mail.mcgill.ca observed in many natural populations, it is more difficult to conclusively link specific environmental factors to specific phenotypic responses. These links may sometimes be more obvious in the case of human-caused environmental disturbances (Baker & Stebbins 1965; Reznick & Ghalambor 2001; Blondel 2008; Hendry, Farrugia & Kinnison 2008) in situations where one aspect of the habitat is manipulated. The present study considers one such possibility in Trinidadian guppies (*Poecilia reticulata*) that show extreme variation in colour patterns. Male guppy colour is a trait subject to both natural and sexual selection, yet polymorphism within and among populations remains high. Despite active research in this system, how particular environmental agents are responsible for the maintenance of this variation is not yet evident (Endler 1995; Brooks 2002).

Phenotypic variation and responses to environmental change may be particularly complicated with regards to secondary sexual traits. Complications arise here because such traits are sensitive to both natural and sexual selection (Darwin 1871; Andersson 1994), often involve multiple correlated traits (Brooks & Couldridge 1999; Candolin 2003), and can show phenotypic plasticity and context dependence (Griffiths & Sheldon 2001; Price 2006). Colour-based secondary sexual traits may be particularly sensitive to environmental change. For instance, colour is responsive to small scale variation in habitat and social interactions (Chunco, Mckinnon & Servedio 2007; Gray & Mckinnon 2007; Roulin & Bize 2007), and so even subtle variation in microhabitats can promote colour change. Larger-scale perturbations should have even larger effects, as suggested by the impact of urbanization, pollution and deforestation on bird plumage (e.g. Eeva, Lehikoinen & Ronka 1998; Horak *et al.* 2001; Smith *et al.* 2008). Other examples can be found in fishes. One is eutrophication of the Baltic Sea which resulted in accelerated algal growth, and a subsequent decrease in visibility for mate signaling. Consequently, the strength of sexual selection for red colouration in sticklebacks decreased; likely due to the increased costs of carrying (males) and choosing (females) bright red colour (Candolin, Salesto & Evers 2007; Engstrom-Ost & Candolin 2008). Similar effects seem to have occurred in the sand goby (Jarvenpaa & Lindstrom 2004) and cichlid fishes (Seehausen, van Alphen & Witte 1997).

Moreover, trait expression and selection can have cascading effects on each other. On the one hand, plastic changes in sexually selected signals can influence both natural and sexual selection, thus influencing their future evolution (West-Eberhard 2005; Price 2006; Svensson & Gosden 2007). On the other hand, sexual selection can change without direct alteration of traits, such as when environmental change alters signal reception and transmission (Endler 1992; Rosenthal 2007; Cockburn, Osmond & Double 2008; Gray *et al.* 2008). To date, however, the impacts of environmental change on components of sexual selection are rarely investigated, although the topic is gaining interest (Svensson & Gosden 2007; Candolin & Heuschele 2008; Cockburn, Osmond & Double 2008).

In this study we take advantage of a population of guppies that recently experienced an environmental disturbance in the form of canopy clearing around the stream. We had fortuitously sampled guppies for 2 years immediately prior to the canopy clearing and were able to sample guppies again for 2 years afterward. We thus consider the disturbance to be an unplanned ‘experiment’ with before and after samples. We also compare samples from the same years at nearby sites that spanned the natural range of canopy cover, but remained relatively undisturbed during the same time period.

Light environment, colour and guppies

With respect to sexual selection in guppies, colourful males are often preferred by females (e.g. Houde 1987; Long & Houde 1989; Houde & Endler 1990; Endler & Houde 1995; Grether 2000; Brooks & Endler 2001a; Pilastro *et al.* 2004). With respect to natural selection, colourful males may be more susceptible to predatory fishes, perhaps because they are more conspicuous (Endler 1978, 1983; Godin & McDonough 2003). Spatial variation in male guppy colour should therefore reflect spatial variation in the relative strengths of opposing natural and sexual selection. Indeed, male guppies in low-predation sites are usually, although not always, more colourful than are male guppies in high-predation sites; the latter often evolving larger and more numerous orange and structural (blues, greens, violets) spots (Haskins *et al.* 1961; Endler 1978, 1980; Millar *et al.* 2006).

Factors other than predation can also potentially influence the evolution and expression of male colour – a fact evident by the extreme variation in male colour among sites within a given predation environment (Houde & Endler 1990; Endler & Houde 1995; Grether 2000; Grether, Hudon & Endler 2001a; Millar *et al.* 2006). Much of this variation remains unaccounted for (Endler 1995; Brooks 2002) but is likely driven by temporal and spatial heterogeneity in microhabitat parameters. At least part of this variation may be due to differences in forest canopy cover (Endler 1995; Grether, Hudon & Millie 1999; Millar *et al.* 2006) and thus the amount of incident light reaching the stream. Fluctuations in light availability have been shown to influence courtship behaviours, the strength of sexual selection acting on visual signals and thus the direction of evolution of colours in other fish species. In general, colour tends to evolve in order to maximize conspicuousness to potential mates with respect to background contrast. For example, the frequency of yellow anal fin morphs of bluefin killifish decreases relative to blue anal fin morphs in tea-stained waters, where yellow is less visible (Fuller & Travis 2004). Similarly, the strength of female preferences for colour signals fluctuate depending on the lighting environment. For example, female three-spined stickleback preferences for male red throat colouration are weaker in tea-stained streams (Boughman 2001).

With respect to guppies, fluctuations in lighting environments are known to affect the perception of visual signals by conspecifics (Endler 1991) and courtship behaviours (Long & Rosenqvist 1998; Gamble *et al.* 2003; Kolluru & Grether

2005). The strength of sexual selection for colour also tends to depend on lighting conditions (Long & Houde 1989) and colour contrast (Endler 1991; Blows, Brooks & Kraft 2003). Furthermore, the high variability among populations in opsin proteins suggest that differences in visual environments may have imposed different directions of sexual selection for colour that maintain guppy colour polymorphism (Archer *et al.* 1987; Hoffmann *et al.* 2007). Together this suggests that variations in lighting environment should be related to spatial variation in colour patterns in guppies, but no previous study has directly examined the influence of temporal or spatial changes in canopy cover on colour.

Some possible outcomes, however, might be suggested from among-site comparisons and from diet manipulations. First, adult guppies might be larger after canopy clearing. This prediction comes from studies showing that sites with more open canopies have greater primary productivity Endler 1993; Grether, Hudon & Millie 1999; Reznick, Butler & Rodd 2001; McKellar, Turcotte & Hendry 2009) and therefore larger and faster-growing guppies (Grether *et al.* 2001b). In addition, increasing diet levels in the laboratory leads to faster growth and larger size at maturity (Arendt & Reznick 2005). Second, canopy clearing may change some aspects of guppy colour. In particular, sites with more open canopies in nature have males with smaller and less black patches (Millar *et al.* 2006). Moreover, increasing diet and carotenoid levels in the lab (both likely associated with more open canopies in nature) leads to males with increased saturation but reduced brightness of orange spots, larger yellow tail spots, and less black colouration (Kodric-Brown 1989; Grether 2000).

Another potential influence of canopy cover is on the spectral properties of incident light, which can change how visual signals are transmitted and received. For example, lower light can cause guppies to court at shorter distances (Endler 1991; Long & Rosenqvist 1998), thus influencing the conspicuousness of colour spots and the responsiveness of females (Endler 1991; Gamble *et al.* 2003). Moreover, canopy cover alters the distribution of wavelengths reaching the stream and can therefore change how female guppies perceive a particular male colour pattern (Endler 1991, 1993). Any of the above effects may then alter selection and thus future evolutionary trajectories of male colour.

Although the above comparative and laboratory studies lend some support to a role of canopy cover on colour variation, it has been difficult to isolate this factor from other co-varying environmental factors in nature. Comparisons within and among sites in relation to the clear-cut event thus provide an opportunity to assess the consequences of canopy clearing on the expression of male colour.

Materials and methods

STUDY SITES

Sampling took place in the Marianne River, located on the north slope of Trinidad's Northern Mountain Range (see Fig. S1 in Supporting Information), at the end of the dry season (March/April) in each of 4 years (2002, 2003, 2006, 2007). Guppies at all sites coexist with only two potential aquatic predators: killifish (*Rivulus hartii*) and freshwater prawns (*Macrobrachium spp.*). Both killifish and prawns have relatively mild effects on guppy demography (Reznick *et al.* 1996), and our study sites are thus categorized as 'low-predation' environments (Haskins *et al.* 1961; Reznick & Endler 1982).

The focal site ('Disturbed') is a low-order stream that we first sampled in 2002 as part of our survey of variation in the Marianne (site 'M16' in Crispo *et al.* 2006; Hendry *et al.* 2006 and Millar *et al.* 2006). We did not visit the site in 2004, but in 2005 we found the forest canopy being cleared by farmers for a papaya plantation. Returning in 2006, we found that the canopy had been completely removed. We therefore sampled guppies from this site in both 2006 and 2007. This enabled a comparison of pre-disturbance samples (2002 and 2003) to post-disturbance samples (2006 and 2007).

In each of the 4 years, we also sampled two 'reference' sites of similar size and stream-order to the disturbed site. These sites represent the extremes of canopy cover among all our sampled low-predation sites in the Marianne and the canopy remained undisturbed throughout the sampling period. These reference sites are used to place temporal changes at the disturbed site in the context of (1) differences between sites with an extreme contrast in canopy cover, and (2) changes through time at sites without recent canopy clearing. See Table S1 in Supporting Information for more details on the specific locations of the sites sampled.

At each of the four sites in 2003 and 2007, we estimated canopy cover by use of a concave spherical densiometer. This method yields readings that are closely correlated with those from hemispherical photography (Englund, O'brien & Clark 2000) and accurately predict relative amounts of standing algal biomass (Grether *et al.* 2001b; McKellar, Turcotte & Hendry 2009). For these readings, each of the sample sites was first divided along its length into 3–6 evenly-spaced transect locations. Spacing between locations was constant at a site but varied among sites (from 5–20 m) to match the local areas from which guppies were sampled. At each location, four densiometer readings were taken (one in each cardinal direction) while standing in the middle of the wetted channel.

GUPPY COLLECTION AND TRAIT MEASUREMENT

Handheld butterfly nets were used to collect at least twenty fish of each sex at each site in each year. These fish were transported live to our laboratory in Trinidad, held for 24–48 h, and then killed with an overdose (40 mg L^{-1}) of tricaine methanosulfate (MS-222). We use MS-222 to immobilize fish for the photographs and also to standardize for behaviourally-plastic effects on colour variation, which are common in fishes. MS-222 generally affects colour by increasing the size of melanophores in all colour patches and making edges of colour patches more distinct (Endler 1991). We have observed that for most colours the average size of spots on individual males is not affected by the anaesthetic (orange: $t_{1,29}$ = 0·05, P = 0·96; structural: $t_{1,29}$ = 0·48, P = 0·63). The only exception is that fish photographed under anaesthetic have larger black spots on average compared to unaesthetized fish ($t_{1,29}$ t = 3·09, P = 0·005) and similarly, brightness of all colour spots is reduced under MS-222 (A. Schwartz & N.P Millar, unpublished data). These results are repeatable across years and consistent among the 13 populations included, and should therefore not bias our results for the present comparative analysis. Nontheless, it is important to remember that colour quantified in this manner is useful for relative comparisons among groups but not for an absolute characterization of what guppies would look like in the field (quantifying colour on free-swimming guppies has not yet been attempted).

Each fish was photographed with a digital camera positioned at a standard height above a grid-ruled background. In 2002, the photographs were taken under natural light in the shade. In all other years, photographs were taken under full-spectrum fluorescent lights that approximate the colour spectrum of sunlight (Vita-Lite by Duro-Test Canada). A set of colour standards was placed in all photographs in 2006 and 2007. Ideally, had we pre-planned this 'experiment', we would have used common lighting conditions and colour standards throughout, and we would also have included more sophisticated analyses, such as spectrometry (Grether, Hudon & Endler 2001a; Grether, Cummings & Hudon 2005; Kemp, Reznick & Grether 2008). This would have been useful because (1) populations can differ in the spectral properties of their colour spots even if they do not differ in the numbers and sizes of those spots (Kemp, Reznick & Grether 2008), and (2) diet can influence the spectral properties of spots (Grether 2000). Such data were not available, however, because our pre-disturbance samples were extracted from a large survey (34 sites in each of 2 years, Millar *et al.* 2006), for which time limitations did not allow spectrometry. Therefore, we rely on the simple and classic methods of counting and measuring the size and number of spots of different colour classes. This method may be biased toward human vision but it is the most broadly comparable to previous work, as nearly all studies report such data (e.g. Endler 1980; Endler & Houde 1995; Grether 2000; Brooks & Endler 2001b; Alexander & Breden 2004; Millar *et al.* 2006), even if they also report spectral properties (Grether, Cummings & Hudon 2005; Kemp, Reznick & Grether 2008).

The digital images were analyzed in random order by a single individual (AKS) who was blind to each fish's site and year of collection. Scion ImageJ (http://rsb.info.nih.gov) was used to perform the following measurements. Standard length was measured from the tip of the snout to the end of the caudal peduncle. Body area was measured by tracing the fish's outline with the 'free-hand' tool. Each colour spot on the body and the tail was then located and assigned to one of the nine colour categories used by Millar *et al.* (2006): black, fuzzy black, orange, red, blue, violet, silver, green, and yellow. The area of each spot was then measured with the free-hand tool, and these areas were summed to yield the total area of a fish covered by each colour category. For all colours except yellow, tail and body spot areas were summed. Repeatability estimates, based on multiple photographs of the same 60 fish, were high: e.g. $r = 0{\cdot}82$ for orange area and $r = 0{\cdot}87$ for black area.

To simplify the analysis and presentation of so many possible response variables, some of which are correlated, we further combined the above nine categories in three more inclusive categories that have particular biological relevance (Endler 1980; Brooks & Endler 2001b; Blows, Brooks & Kraft 2003). These categories were black (includes black and fuzzy black), carotenoid- and pteridine-based (red, orange, yellow), and structural (blue, violet, silver & green). 'Colour categories' in the rest of this paper refer to these three inclusive categories. We also analyzed yellow tail area separately because most yellow colour is found on the tail and this trait is very sensitive to diet manipulation in the laboratory (Grether 2000).

STATISTICAL ANALYSIS

Our main goal was to determine if the disturbance event (canopy clearing) influenced phenotypic variation at the disturbed site. Phenotypic changes between sampling periods (2002–2003 vs. 2006–2007) at the disturbed site but not at the reference sites would suggest that some aspect of the disturbance was responsible. Furthermore, if changes at the disturbed site are in the direction of differences between the closed-canopy and open-canopy reference sites, then canopy cover could be implicated as the specific cause. We addressed these questions through analyses of female and male size (standard length) and the area of different colour categories on males. Analyses of colour area controlled for allometry by using the residuals of colour area on total body area. Each of the variables was analyzed individually (ANOVAS) and colour variables were also analyzed jointly (MANOVA).

We first analyzed variation among all our samples in two-way models with the following structure: sampling period (2002–2003 vs. 2006–2007), year (nested within sampling period), and site. We then analyzed pair-wise variation between sites before and after the clear-cut event. Statistical analyses were performed in JMP for Macintosh (v.7.02. SAS Institute, Cary, NC, USA).

Results

ENVIRONMENTAL CHANGE AT THE DISTURBED SITE

Canopy openness at the disturbed site was 22·19% (± 1·11 SE) in 2003 and increased to 69·68% (± 7·54) in 2007. This change should increase incident light and therefore primary productivity and food availability (Grether *et al.* 2001b). We could not directly test for such cascading effects at the disturbed site because we did not sample algae before the disturbance. However, we have indirect evidence from samples of algal standing crop collected in 2007. In that year, the disturbed site had higher levels of chlorophyll *a* than did all 22 of the other Marianne and Paria sites (McKellar, Turcotte & Hendry 2009), wherein a strong positive correlation was found between canopy cover and productivity. We therefore suggest that the apparent increase in productivity at the disturbed site was the result of increased incident light. An alternative (agriculture fertilizers, for example) was unlikely given the low agricultural activity intensity - it only continues for 50 m upstream of our study site.

Over the same time period (2003 vs. 2007), the reference sites showed much less change in canopy cover. At the closed reference site, canopy openness remained essentially constant: 12·17% (± 0·47 SE) in 2003 and 7·37% (± 0·72 SE) in 2007. At the open reference site, canopy openness appeared to decrease from 87·05% (± 1·83 SE) in 2003 to 52·65% (± 6·53 SE) in 2007, but also varies more substantially across the length of the stream (see Table S1 in Supplementary Information for full details). Regardless, the key point is that the two reference sites differed dramatically in canopy cover, and that the disturbed site changed through time along this same continuum.

PATTERNS OF PHENOTYPIC VARIATION

Both male and female body size increased from the first (2002–2003) to the second (2006–2007) sampling period at the disturbed site but not at the reference sites (Table 1, Fig. 1).

Table 1. Overall effects of sampling period (2002–2003 vs. 2006–2007), year (nested within sampling period), and site (Open, Closed, Disturbed) on phenotypic variation. Shown are *F*-ratios with significance indicated by asterisks

Factor d.f.	Sampling period 1/243	Year 2/243	Site 2/243	Sampling period × Site 2/243
Female length	4·65*	3·1*	**15·01******	11·41****
Male length	10·14*	3·1*	**98·92******	16·36****
Male colour (MANOVA)	4·29**	2·5*	**7·82******	1·1
Orange area	2·23	0·05	**12·0******	0·35
Yellow tail area	2·67	2·1	**3·73***	0·74
Black area	2·47	1·57	**4·65***	1·34
Structural area	0·44	5·48**	**7·27*****	1·72

*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$; ****$P < 0·0001$. Factors with the largest effects are shown in bold for each trait.

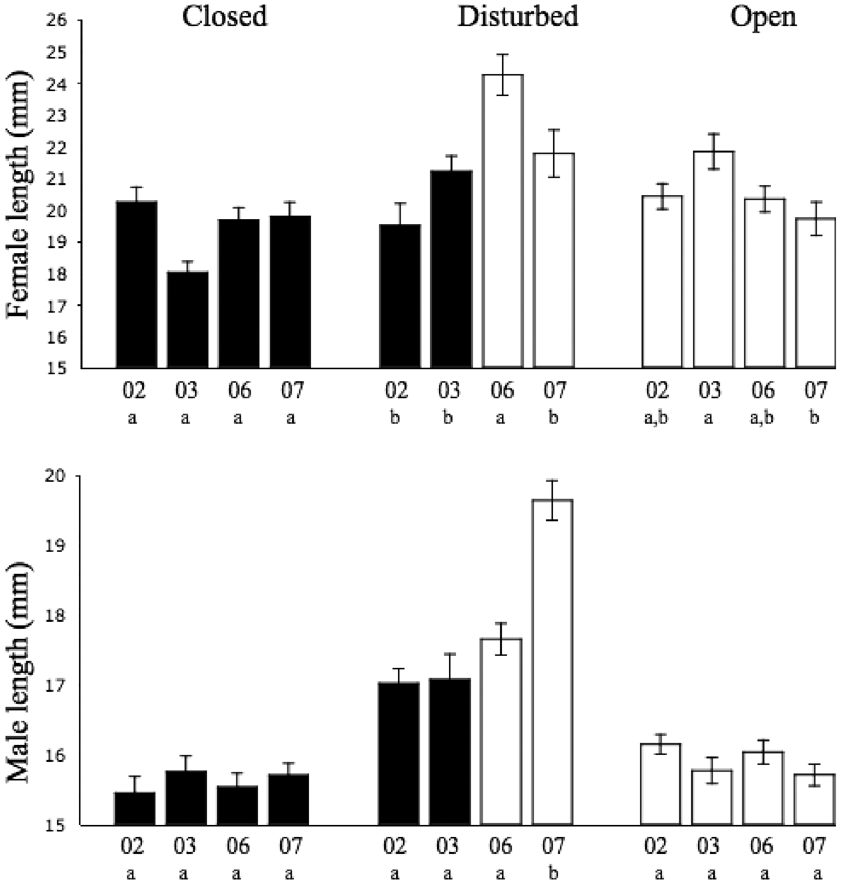

Fig. 1. Means and standard errors for male and female size (standard length). 'Closed' = closed canopy reference site. 'Open' = open canopy reference site. 'Disturbed' = site where canopy clearing occurred between March of 2005 and March of 2006. Solid bars indicate closed-canopy samples and open bars indicate open-canopy samples. Letters below years indicate homogeneous subsets (*post hoc* Tukey tests) comparing variation among years *within* each site.

As a qualification, however, note that only one of the two post-disturbance samples showed a substantial size difference from the pre-disturbance samples for each of the sexes (Fig. 1). Overall, fish were larger at the open reference site (females: $F_{1,248} = 16·63$, $P < 0·0001$; males: $F_{1,168} = 4·74$, $P = 0·03$), however the magnitude of this difference varied throughout the sampling period: no differences in size were detectable between reference sites for males within the first and second sampling periods, whereas females were significantly larger at the open reference site in the first, but not the second, sampling period (Table 2, Fig. 1). When comparing the disturbed and reference sites, guppies at the former were larger in most cases, although the differences were always largest after the disturbance (Table 2, Fig. 1). That is, although guppies were somewhat larger at the disturbed site before canopy clearing, they were considerably more so after canopy clearing.

None of the colour variables showed consistent, statistically significant differences between the first and second sampling periods at any of the sites (Tables 1 and 2, Fig. 2). Instead, male colour varied considerably among sampling sites (Table 1, Fig. 2) but this was generally independent of both the disturbance event and differences in canopy cover among sites. Males at the two

Table 2. Pairwise comparisons between sites before (2002–03) and after (2006–2007) the clear-cutting event at the disturbed site. Shown are *F*-ratios from one-way analysis of variance with significance indicated by asterisks

	Open vs. Closed		Open vs. Disturbed		Closed vs. Disturbed	
Year	02–03	06–07	02–03	06–07	02–03	06–07
d.f. (females)	1/76	1/81	1/78	1/91	1/76	1/88
d.f. (males)	1/78	1/108	1/78	1/84	1/78	1/84
Female length	15·23*** O > C	0·468	2·71	23·78**** D > O	5·85* D > C	28·76**** D > C
Male length	3·18	1·63	16·33*** D > O	122·62**** D > O	30·18**** D > C	133·93**** D > C
Male colour (MANOVA)	1·77	3·24*	12·04****	4·54*	6·11**	4·05*
Orange area	0·25	0·17	9·89** D < O	8·07** D < O	11·56** D < C	7·06** D < C
Yellow tail	0·08	0·97	0·93	2·05	1·34	6·52* D > C
Black area	4·54* O < C	6·72* O < C	4·29* D > O	0·44	0·10	2·94
Structural area	8·92** O < C	0·19	16·24*** D > O	1·43	2·51	2·11

$*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0.001$; $****P < 0{\cdot}0001$. The direction of the difference is indicated for significant differences (O, open; C, closed; D, disturbed).

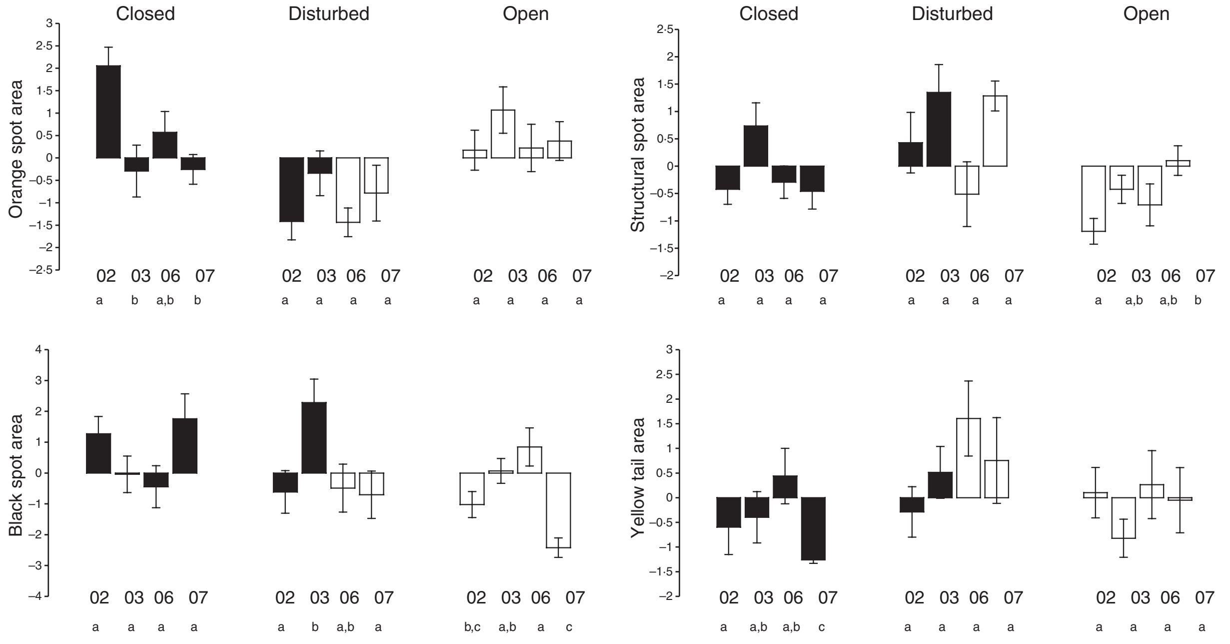

Fig. 2. Means and standard errors of residuals (from body area) of the area of colour spots in different categories. See the caption for Fig. 1 for labelling and other conventions.

reference sites did differ somewhat in some aspects of colour, however these differences were not temporally stable. The closed reference site had males with more black in both sampling periods and less structural colour in the first sampling period (Table 2, Fig. 2). Males at the disturbed site also differed from males at the two reference sites: the disturbed site had males with more black compared to the open reference site, although only prior to canopy clearing. Moreover, males at the disturbed site had the least amount of orange colouration throughout the sampling period.

Temporal variation in site differences were evident for the other aspects of colour. Specifically, males in the disturbed site had more yellow on their tails relative to males at the closed site following the clear-cut only. Prior to the clear-cut, males at the disturbed site had significantly more black and structural colour than did males at the open site (Table 2, Fig. 2).

Discussion

We explored temporal and spatial variation in guppy body size and male colour in relation to (1) a recent disturbance event that dramatically reduced canopy cover at a single location ('disturbed' site), and (2) longer standing variation in canopy cover ('reference' sites). Because the appearance of colour patterns has been shown to vary among lighting environments (Endler 1991), and changes in mating behaviour have been associated with variations in resource and light availability (Gamble *et al.* 2003; Kolluru, Grether & Contreras 2007), variations in canopy cover is expected to affect phenotypic variation.

Although we found that canopy clearing generally led to larger guppies (Fig. 1; Table 2), consistent with the expectation that sites with more open canopies have more resources for guppies which then enhances their growth (Grether *et al.* 2001b; Karino & Haijima 2004; Arendt & Reznick 2005), there was no consistent effect of canopy cover on colour variation. One exception is more open canopies were, to some extent, associated with less black colour for a given male body size (Fig. 2; Table 2). That is, males had less black at the open canopy reference site than at the closed canopy reference site and males had less black at the disturbed site than at the open canopy site before, but not after, canopy clearing. This trend further fits with expectations from previous work based on among-site comparisons in the Marianne River (Millar *et al.* 2006) and diet manipulations in the laboratory (Grether 2000).

Overall, however, among-site variation independent of canopy cover proved very important in explaining variability in male colour (Table 1). Perhaps most striking, males at the disturbed site, both before and after the disturbance, had less orange and more structural colour, than did males at both reference sites (Fig. 2). In addition to spatial variation, we documented considerable temporal variation at a given site, even when predation regime or canopy cover did not change appreciably.

These results are in stark contrast to expectations for guppies and other fish species that show colour polymorphism. In guppies, fluctuations in light environments change the way that colours are perceived by conspecifics (potential mates) and heterospecifics (potential predators). Specifically, colour brightness and contrast evolves to maximize conspicuousness to mates and minimize conspicuousness to predators within a given lighting environment (Endler 1983, 1991; Archer *et al.* 1987). Furthermore, mating preferences for orange colour tend to decrease when ambient light conditions go from blue/white (lighting conditions representative of relatively open canopies) to reddish/brown (lighting representative of relatively closed canopies) (Long & Houde 1989; Endler 1991). In other species, similar effects have been observed (e.g. stickleback, Boughman 2001; killifish, Fuller & Travis 2004), but unlike the current study, such effects of sexual selection often result in a correlation between phenotypic variation and variation in ambient light and water transmission properties (see Boughman 2002 for review).

Why then, did guppies in the Marianne not show the same response to such extreme fluctuations in ambient light? One potential difference between guppies and other fish species is that variation in guppy colour is continuous, rather than consisting of two or more morphs (e.g. cichlids, killifish) and is a multicomponent signal, consisting of up to 8 or 9 different colour patches on a single individual rather than continuous variation in a single colour, (e.g. red throat colour of stickleback). Because most individual guppies have all colours, it is also their position to one another that constitutes an individual colour pattern, not only their relative sizes. Natural and sexual selection both likely target maximizing visual contrast between colour patches, however the present study demonstrates that light is not the only selective force shaping variation in colour perception.

Instead, temporal, and particularly spatial variation in colour was high among our three study sites, regardless of proximity and canopy cover. One reason for spatial variation might be unmeasured environmental parameters, such as parasite loads (e.g. Houde & Torio 1992; but see Martin & Johnsen 2007) and the number of red-blind *Macrobrachium* prawns (Endler 1978, 1991; Kemp, Reznick & Grether 2008; NP Millar, unpublished data). Another reason may be among-site variation in female choice for male colour (e.g. Houde & Endler 1990; Endler & Houde 1995; Schwartz & Hendry 2007). Indeed, recent studies in several taxa are increasingly pointing to the importance of spatial variation in sexual selection, instead of just natural selection, as a driver of spatial variation in phenotypes (Kwiatkowski & Sullivan 2002; Roulin & Bize 2007; Gosden & Svensson 2008). Of course, spatial variation in sexual selection might be itself the result of spatial variation in environmental parameters. For example, the direction of sexual selection on particular colours may be influenced by variation in the visual background (Endler 1978, 1991) or the presence/absence of various predators (Breden & Stoner 1987; Stoner & Breden 1988; Houde & Endler 1990; Schwartz & Hendry 2007). Alternatively, sexual selection may diverge among populations in arbitrary directions owing to founder effects (e.g. Carlson 1997; Gavrilets & Boake 1998), drift (Lande 1981), or population-specific sensory biases (reviewed in Endler & Basolo 1998).

One potential factor contributing to temporal variation in colour may be related to the movement of fish between sites in a stream, given that a number of studies have shown guppies can move substantially over time (Croft *et al.* 2003; Barson, Cable & van Oosterhout 2009). Such movement may be passive, for example due to flooding in the rainy season, or active, such as to increase foraging or mating opportunities. These regular changes in the composition of a population may help to maintain variation, particularly since males with rare colour patterns appear to have an advantage in terms of predation (Olendorf *et al.* 2006) and mate choice (Eakley & Houde 2004; Zajitschek & Brooks 2008).

IMPLICATIONS FOR MATE CHOICE AND SEXUAL SELECTION

Most colour pattern elements did not show consistent associations with canopy cover. This result might imply that canopy clearing will not influence sexual selection on male colour but this is not necessary so. For example, the increase in fish size under open canopies may alter the visibility or attractiveness of particular colour patterns (even though the relative amount of colour spots themselves do not change), thus influencing the degree of contrast with the background or other colour spots. In addition, canopy clearing changes the light environment and therefore influences the manner in which females perceive and respond to males (Gamble *et al.* 2003; Kolluru, Grether & Contreras 2007). Males tend to court most readily at dawn and dusk, when lighting conditions favour conspicousness to the female guppy visual system, but not the visual system of potential predators (Endler 1987, 1991). Changes in lighting conditions may therefore evoke changes in behaviour, rather than selection on colour elements in particular.

Furthermore, the increase in productivity at our focal site may, for example, have provided better foraging opportunities that could influence the benefits to mate choice (Grether, Cummings & Hudon 2005). In particular, female guppies often prefer males with larger and more saturated orange spots (Houde 1987; Kodric-Brown 1989; Grether 2000; Brooks & Endler 2001a; Blows, Brooks & Kraft 2003), perhaps because large areas of saturated orange reflect male quality through foraging ability (Nicoletto 1991; Houde & Torio 1992; Godin & Dugatkin 1996; Grether *et al.* 2004; Locatello *et al.* 2006) - the ability to saturate orange spots depends on the uptake of carotenoids and other pigments from the diet (Grether 2000). Sites with more open canopies have greater carotenoid availability and could allow even males of genetically-poor quality to optimally saturate large areas of orange. If so, we might expect a reduction in the strength of female preferences for this trait, ultimately weakening the influence of sexual selection on this aspect of orange colour. Although we did not measure the saturation of orange spots, canopy clearing events may provide an opportunity to test this aspect of the indicator hypothesis.

The results of this study demonstrate that fluctuations in lighting environment alone do not mediate polymorphism in sexually selected signals. Instead, a combination of environmental factors that affect visual perception are likely important determinants, as well as the extent to which female preferences can respond to such environmental fluctuations. Chunco *et al.* (2007) formalized this hypothesis with a population genetic model that showed the strong influence of the inheritance mode of preferences on the maintenance of colour polymorphisms. Specifically, if females inherit preferences from their fathers, sexual selection is more likely to maintain polymorphism, even without environmental heterogeneity. Stronger knowledge of the genetics of colour perception is likely the next challenge toward our understanding of colour variation both in the guppy system and beyond.

FUTURE DIRECTIONS: RE-EXAMINING GUPPY COLOUR

Our baseline data provide the opportunity for an assessment of future evolutionary responses to canopy clearing. Our results indicate that differences in light levels do not appear to be causing changes in the variation in basic colour categories, but may nonetheless influence the mechanisms and strengths of mate choice (as above). Little is known about the rate of evolution of female preferences in natural populations, but such traits might take more time to produce a phenotypic response in males. Our study can therefore be viewed as stepping-stone for further work on the influence of human-caused perturbations on the expression and evolution of sexual signals. Will male colour evolve to a new state, as might be expected if natural or sexual selection has been altered? Will female preferences evolve and, if so, will they evolve in response to changes in canopy cover or to the male traits? What will happen if the canopy is allowed to grow back?

Such work would be particularly informative in a general sense because few studies have examined the contemporary evolution of sexually selected traits (Svensson & Gosden 2007), although it is likely that their responses may be qualitatively different from traits subject only to natural selection (Hoekstra *et al.* 2001; Kingsolver *et al.* 2001). Trinidadian guppies may be an excellent model of such a circumstance. Despite the long history of research on colour in Trinidadian guppies, recent studies are increasingly pointing to the need for a closer look. Although life-history trait evolution has been unmistakably and repeatably attributed to differences in mortality rates due to predation (Reznick *et al.* 1997; Gordon *et al.* 2009), and differences in canopy cover (Grether *et al.* 2001b; Arendt & Reznick 2005), the effect of the same broad predation differences on colour evolution is less consistent. The present study demonstrates considerable spatial and temporal variation in the colour of low-predation males in a single river that is not predictable based on differences in canopy. Due to the multiple agents interacting on the maintenance and perception of colour elements in both conspecific and heterospecific interactions, it appears that understanding the evolution of guppy colour will require a further quantification of habitat and demographic variation that goes beyond dichotomous (e.g. high vs. low predation; open vs. closed canopy) environmental parameters.

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Supporting information

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

Fig. S1. Map of study sites in Trinidad's Northern Mountain Range.

Table S1. Site locations and temporal variation in canopy cover.