



Using adaptive traits to consider potential consequences of temporal variation in selection: male guppy colour through time and space

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Temporal variation in selection is typically evaluated by estimating and comparing selection coefficients in natural populations. Meta-analyses of these coefficients have yielded important insights, but selection coefficients are limited in several respects, including low statistical power, imperfect fitness surrogates, and uncertainty regarding consequences for trait change. A complementary approach without these limitations is to examine temporal variation in adaptive traits themselves, which is mechanistically easier and more directly relevant to evolutionary consequences. We illustrate this approach by analyzing the colour patterns of male guppies, *Poecilia reticulata*, from each of six sites in Trinidad in each of 6 years. This system is particularly appropriate for our study because key aspects of colour variation are genetically-based and responsive to selection. However, although spatial patterns of colour variation have been extensively considered in this system, no study has yet formally assessed annual temporal variation in non-manipulated populations. Matching previous conclusions for the guppy system, we find that guppies from different sites manifest different colour patterns in association with different predation regimes. We here add the new finding that, although some temporal variation is present, spatial patterns of colour variation are generally consistent across years. These results suggest that, when considering adaptive traits, spatial variation is more important than temporal variation, although our study system might be exceptional in this regard. Additional studies examining spatiotemporal variation in adaptive traits could help to improve our understanding of the role that spatiotemporal variation in selection plays in the evolutionary process. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **112**, 108–122.

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INTRODUCTION

The extent to which selection acting on natural populations is temporally consistent or variable has been the subject of several recent meta-analyses of published selection coefficients, but the answer remains unresolved (Siepielski, DiBattista & Carlson, 2009; Bell, 2010; Kingsolver *et al.*, 2012; Morrissey & Hadfield, 2012). Some meta-analyses suggest that substantial temporal variation is present (Siepielski *et al.*, 2009; Kingsolver *et al.*, 2012), whereas others

suggest that apparent temporal variation is an artefact of sampling error (Morrissey & Hadfield, 2012). It will be hard to conclusively resolve this question on the basis of selection coefficients alone because it is very difficult to accurately and precisely measure selection in natural populations (Kingsolver *et al.*, 2001; Hereford, Hansen & Houle, 2004; Hersch & Phillips, 2004; Morrissey, Kruuk & Wilson, 2010). Furthermore, selection coefficients cannot, by themselves, resolve the critical question of whether or not temporal variation in selection has important evolutionary consequences for adaptive traits.

A complementary approach that avoids the above limitations of selection coefficients is to directly examine temporal variation in the adaptive traits

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themselves (Grant & Grant, 2002; Aguirre & Bell, 2012), although this approach has caveats of its own (see Discussion). One advantage is that measurement of phenotypic trait values is easier and subject to less bias than is the estimation of selection coefficients. The reason is that estimating selection also requires accurately estimating fitness, which can be a difficult undertaking for both practical and theoretical reasons, especially in natural populations (Kingsolver *et al.*, 2001; Hereford *et al.*, 2004; Hersch & Phillips, 2004; Morrissey *et al.*, 2010). Indeed, a number of studies have found that selection coefficients are often not predictive of evolutionary change (Merilä, Sheldon & Kruuk, 2001) and that estimates of selection in well-adapted populations will not reflect the selection that drove that adaptation in the first place (i.e. selection erases its traces; Haller & Hendry, 2014). Thus, another advantage of monitoring adaptive traits themselves is that they point more directly to any realized *consequences* of temporal variation in selection. Although temporal variation in traits will not necessarily match temporal variation in selection, it should provide a valuable and complementary contribution to discussions about the importance of temporal variation in the evolutionary process. We emphasize that we are not advocating a replacement for estimating selection on adaptive traits, nor are we dismissing the valuable contributions selection estimates can provide to understanding adaptation. Rather, we are suggesting a complementary approach for gaining further insight into the importance of temporal variation in selection.

We further suggest that a comparative approach would be especially useful for evaluating the importance of temporal variation (in selection or adaptive traits). That is, we should be asking how important temporal variation is in relation to other aspects of variation, with the most obvious candidate being spatial variation among conspecific populations (Blanckenhorn *et al.*, 1999; Gosden & Svensson, 2008; Weese *et al.*, 2010; Sternalski, Mougeot & Bretagnolle, 2012; Cox & Rabosky, 2013). It is generally accepted that spatial variation in selection, and hence adaptation, is very strong (Endler, 1986; Schluter, 2000), which provides a useful basis for assessing the importance of temporal variation. If, for example, temporal variation is as strong as spatial variation, we can safely assume that both are important in the evolutionary process. In some situations, temporal variation might be synchronized across a number of populations (e.g. through regional climate forcing), in which case the main effect of time would be of interest. In most situations, however, temporal variation will be asynchronous across space (e.g. through local fluctuations in biotic and/or abiotic conditions), in which case the interaction between space and time

would be of greatest interest. That is, the key question becomes: how consistent through time are the spatial patterns of variation?

STUDY SYSTEM

We implement the above ideas through an analysis of spatiotemporal variation in the colour patterns of male Trinidadian guppies, *Poecilia reticulata* (Peters). This system is appropriate for several reasons. First, guppy colour is known to differ among populations in response to spatial variation in selective pressures, such as predation (Endler, 1980; Magurran, 2005), resource levels (Kodric-Brown, 1989; Grether, Hudon & Endler, 2001a; Millar *et al.*, 2006; Schwartz & Hendry, 2010), parasites (Houde & Torio, 1992), and sexual selection (Endler & Houde, 1995; Houde, 1997; Schwartz & Hendry, 2007). In general, sexual selection by females results in positive selection for conspicuous colour such as orange (Houde, 1997), whereas predation by visual predators results in negative selection conspicuous colours (Endler, 1980; Magurran, 2005). This spatial variation in colour provides a useful benchmark for considering the effects of temporal variation. Second, although some aspects of guppy colour, such as carotenoid deposition, brightness, and chroma, are influenced by plasticity (Grether *et al.*, 2001a; Grether, Cummings & Hudon, 2005; Miller & Brooks, 2005; Ruell *et al.*, 2013), the size, number, and location of colour spots is highly heritable and differences within and among populations are mostly genetically based (Houde, 1992; Brooks & Endler, 2001; Karino & Haijima, 2001; Hughes, Rodd & Reznick, 2005; Tripathi *et al.*, 2009; Gordon, López-Sepulcre & Reznick, 2012). These properties increase the likelihood that spatiotemporal variation in the colour properties documented in the present study reflects spatiotemporal variation in underlying genotypes; however, with a field study such as ours, we cannot be certain to have completely eliminated plastic effects. Third, the generation length of guppies is short enough (approximately 1.8 generations per year; Reznick *et al.*, 1997) that temporal variation in selection could drive adaptive genetic change, and thus phenotypic change, on a yearly time scale (Endler, 1980).

Studies analyzing spatial variation in traits often replicate their samples in at least 2 years (examples in supplementary data from Siepielski *et al.*, 2013) to confirm temporal consistency of the observed patterns. Remarkably, none of the many studies of male guppy colour in non-manipulated populations has yet replicated their samples across multiple years. Instead, the few studies with replicates across years have focused on the effects of major human-caused disturbances, such as introduction to a new predation

regime (Endler, 1980; Karim *et al.*, 2007; Kemp *et al.*, 2009), introduction to a new locality (Kudo & Karino, 2013) or the clear-cutting of riparian vegetation (Schwartz & Hendry, 2010). As a result, the extent of temporal variation in male guppy colour remains unknown, although such variation appears to be possible. In particular, each of the above-mentioned selective agents (predation, resource levels, parasitism, and sexual selection) likely varies through time in response to temporal variation in flooding (van Oosterhout *et al.*, 2007; Weese *et al.*, 2011), population density (Reznick, 1989; Reznick, Bryant & Bashey, 2002), sex ratio (Rodd & Reznick, 1997; Pettersson *et al.*, 2004; McKellar, Turcotte & Hendry, 2009), and parasitism (van Oosterhout *et al.*, 2007; Gotanda *et al.*, 2013). Moreover, a mark–recapture study found some evidence of spatiotemporal variation in viability selection on male guppy colour, although that study was attended by the above mentioned concerns surrounding selection estimates (Weese *et al.*, 2010).

In the present study, we assess spatiotemporal variation in male guppy colour through the analysis of photographs from field collections in each of six consecutive years (approximately 10 guppy generations) at each of six different sites in Trinidad. Using genetically-based and known-adaptive colour metrics (sizes and numbers of spots of different colours) that have been reported in many previous studies, we partition the variation among effects of site, year, and their interaction. We then focus our interpretation on the extent to which spatial patterns of variation in guppy colour are consistent through time.

MATERIAL AND METHODS

Each year from 2005 to 2010, live guppies were sampled from each of six different sites (see Supporting information, Fig. S1) along the Marianne and Paria rivers of northern Trinidad. For each year, these samples were always taken during the dry season in the months of February and March. Based on our previous work in these watersheds (Crispo *et al.*, 2006; Hendry *et al.*, 2006; Millar *et al.*, 2006; Schwartz & Hendry, 2010; Weese *et al.*, 2010), we selected sites that (1) were far enough apart so that dispersal between them was minimal making them effectively independent sites for this study; (2) represented a range of different environments so that adaptive spatial variation would be expected; and (3) had large population sizes so that genetic drift would be minimal. In accordance with standard practice, we classified sites as high-predation (HP) or low-predation (LP) according to whether highly piscivorous fishes were present or absent, respectively (Endler, 1980; Rodd & Reznick, 1997; Martin & Johnsen, 2007). On the Marianne River, two sites (M7 and M14) were HP

and two sites (M4 and M16) were LP. On the Paria River, both sites (P4 and P7) were LP, as is the case for all sites in the Paria (Millar *et al.*, 2006, fig. 1; see also Supporting information, Fig S1).

At each site in each year, we used butterfly nets to capture 16–21 male guppies ($N = 20$ for most), except one sample that, as a result of logistical constraints, had only six males (see Supporting information, Tables S1, S2). The fish were transported to our field station in Trinidad, where they were euthanized with an overdose of tricaine methanesulfonate (Finquel MS-222; Argent Laboratories Group) buffered with NaHCO_3 to obtain a neutral pH. This process helps standardize some of the plastic components of male guppy colour (Endler, 1991). Immediately after euthanasia, each guppy was placed left-side-up on a light background and blotted with a paper towel to reduce reflective glare. A digital camera was situated directly overhead and illumination was provided by two full spectrum fluorescent bulbs (Coralife 18" 10 000 K Daylight). Two photographs were taken of each guppy, one with and one without a flash, to be compared side-by-side when collecting colour data (Millar *et al.*, 2006; Karim *et al.*, 2007; Millar & Hendry, 2012). An acrylic colour standard and a ruler were visible in each photograph.

Our analyses of colour variation were based on the size and number of colour spots as inferred from the photographs. This method of analysis represents the classic and most common approach, which means that our results are directly relevant to previous work (Endler, 1980; Kodric-Brown, 1985; Houde & Endler, 1990; Pilastro *et al.*, 2004; Millar *et al.*, 2006; Weese *et al.*, 2010). Moreover, analyses based on these metrics have yielded many robust conclusions regarding the role of natural and sexual selection in shaping guppy colour (Endler, 1980; Houde, 1997; Magurran, 2005). We would ideally also have included analyses based on spectrophotometry and visual modelling (Grether *et al.*, 2001a, 2005; Endler & Mielke, 2005; Kemp, Reznick & Grether, 2008; Kemp *et al.*, 2009), which provides information on how colours are perceived by guppies and their predators (Kemp *et al.*, 2008, 2009). Although we collected spectrophotometric data in 2009 and 2010, these data were not available for prior years and were not analyzed in the present study because our goal was to consider variation in an adaptive trait over a longer time frame.

Since 2008, the photographs were captured in both RAW and JPEG formats, with the former being preferable for colour analysis (Stevens *et al.*, 2007). However, only JPEGs had been captured prior to 2008, and so only JPEGs were used for the present analysis, as has also been the case in other guppy studies (Miller & Brooks, 2005; Pitcher, Rodd & Rowe, 2007; Kudo & Karino, 2012; Kudo & Karino, 2013). All photographs

were taken using the highest possible resolution available for the camera. One individual (KMG), who was blind to the year and site of origin of each guppy, analyzed all 695 photographs in random order. Each photograph was imported as a raster image into MAPINFO PROFESSIONAL, version 6 (Pitney Bowes Software). Individual spot colours were then classified as black, fuzzy black, orange (includes red), yellow, violet, silver, blue, and green (*sensu* Endler, 1978; Endler, 1991; Millar *et al.*, 2006; Karim *et al.*, 2007). Flash and nonflash photographs were compared side-by-side to prevent ambiguity in colour perception (Millar *et al.*, 2006; Karim *et al.*, 2007; Millar & Hendry, 2012), and a colour standard was used as a visual reference in all photographs. Data were collected on the flash photographs. Each spot on the body of the fish (i.e. fins were excluded) was individually outlined in MAPINFO and its area (mm²) was recorded. These areas were summed across all spots of a given colour on a given fish. The resulting 'total area' of each colour on each fish was then divided by the total body area of the fish (again excluding fins) to yield the 'relative area' of each colour. In addition, the number of spots in each colour category was recorded to yield 'spot number' for each guppy. Previous work has shown that these measurements are highly repeatable (Gotanda *et al.*, 2013).

To reduce the number of variables for analysis, the above colours were grouped into biologically relevant categories. These groups have different physiological bases, structural bases, functional interpretations, and selective relevance (Endler, 1978; Kodric-Brown, 1989; Hearing, 1993; Brooks & Endler, 2001; Grether *et al.*, 2001a, 2001b, 2005; McGraw *et al.*, 2002; Blows, Brooks & Kraft, 2003; Griffith, Parker & Olson, 2006). 'Carotenoid colours' consisted of the sum of orange and yellow spots, although note that pigments other than carotenoids also contribute to these spots (Grether *et al.*, 2001a, 2005). 'Structural colours' are colours that are iridescent and have higher levels of reflection, and consisted of the sum of blue, violet, and silver spots (Endler, 1978; Brooks & Endler, 2001). 'Melanic colours' consisted of the sum of black and fuzzy black spots. In addition, green was retained in the analysis as a separate colour category (Weese *et al.*, 2010). To meet statistical assumptions of normality, data were transformed as appropriate.

All data analyses were conducted in R, version 2.15.2, R Core Team, 2012) and involved multivariate and univariate general linear models with year (2005–2010), site (M4, M7, M14, M16, P4, and P7), the year-by-site interaction, and body size as a covariate. In these models, site was clearly a fixed effect because the different sites were specifically chosen for their properties as described above. By contrast, year can be considered to be a random effect

because the specific years should be considered a random selection of six consecutive years. We therefore assessed statistical significance on the basis of mixed models. However, our primary goal was to directly compare the effects and effect size of site, year, and their interaction in a single model, and so we additionally analyzed models with year specified as a fixed effect. Statistical results were similar between the two approaches (i.e. year as fixed or random), and so we only report the fixed-effects analysis (mixed model results appears in the Supporting information, Table S8). In addition, order of entry of terms into the model did not alter conclusions, and so we report models where the site term was fit first.

In each case, we first fit multivariate analyses of covariance with all colour categories (carotenoid colour, structural colour, melanic colour, and green) considered simultaneously as response variables. We then fit separate analyses of covariance for each colour category. All of these models were fit separately for two different classes of colour metrics: relative areas (arcsine square root transformed) and spot numbers (square root transformed), as described above. The size of individual spots was not analyzed because it was statistically redundant to relative areas and spot numbers. Post-hoc Tukey's honestly significant difference tests were run following the analyses of covariance to determine which of the sites differed from each other for each colour category. Effect sizes for the fixed effect models were calculated as partial η^2 based on Wilk's partial η^2 for multivariate tests and sums of squares for the univariate tests (Langerhans & DeWitt, 2004) using the heplot package in R. Ranges for partial η^2 were generated when the data were jack-knifed by omitting one row of data at a time and repeating this with all rows (Langerhans & DeWitt, 2004).

For the interpretation of effects from the above models, first, the site term reflects consistent (across years) spatial variation in colour patterns, which we expect from previous work to be a consequence of spatial variation in selection. Second, the year term reflects consistent (across sites) temporal variation in colour pattern. We assume this variation is a consequence of improvements of both our photographic methods and camera/flash equipment through time rather than any biologically significant regional trends in colour (see Results). Third, the site-by-year interaction term reflects differences between years in the patterns of spatial variation (e.g. guppies from one site are more colourful than those from another site in some years but not other years). This variation would be consistent with temporal variation in selection that differed between sites, although other factors could also contribute (see Discussion.)

Table 1. Results from multivariate analyses of covariance using Wilk's lambda test statistic with site fitted before year as fixed effects and body size (mm²; log transformed) as the covariate

	<i>F</i>	d.f.	<i>P</i>	Wilk's partial η^2
Relative area colour				
Site	36.106	202 173	< 0.001	0.176 (0.175–0.178)
Year	30.040	202 173	< 0.001	0.173 (0.171–0.176)
Year-by-site	2.892	1 002 600	< 0.001	0.099 (0.097–0.102)
Body size	20.224	4 655	< 0.001	0.084 (0.079–0.087)
Spot number				
Site	25.451	202 173	< 0.001	0.135 (0.133–0.138)
Year	15.339	202 173	< 0.001	0.096 (0.095–0.097)
Year-by-site	2.362	1 002 600	< 0.001	0.083 (0.081–0.084)
Body size	12.594	4 655	< 0.001	0.081 (0.075–0.085)

Estimates of variance were calculated as multivariate partial η^2 also utilizing Wilk's lambda. Numbers in parentheses are the ranges generated when the data were jack-knifed. The dependent variables were relative area (arcsine square root transformed) and spot number (square root transformed) for all colour categories (carotenoid colour, structural colour, melanic colour, and green). Statistical significance ($P < 0.05$) is indicated in bold.

Finally, we placed our results in the context of an earlier, single-year survey that included our six sites and 23 additional sites (Millar *et al.*, 2006). That earlier study found a strong negative association across populations between mean relative areas of orange and mean relative areas of blue. We therefore used Pearson product-moment correlations to test, in the present data set, for the same relationship among all years and within each year.

RESULTS

Multivariate analyses of relative colour area and of spot number revealed significant effects of all the independent variables, body size, site, year, and the year-by-site interaction, in all cases (Table 1). Of these effects, site and year explained the most variance in relative colour area and site explained the most variance in spot number (Table 1). Relative to site, the year-by-site interaction explained 44% as much of the variation (difference between the larger and smaller value divided by the larger value) in relative colour area and 39% as much of the variation in spot number (Table 1).

Univariate analyses revealed highly significant effects of all the independent variables on most colour categories and colour metrics (Table 2). Site explained the most variance in carotenoid colour relative area and spot number, structural colour relative area and spot number, and melanic colour spot number; for carotenoid colour spot number, year explained almost the same amount. Year explained the most variance for the other colour variables, although site explained almost as much for green relative area. Relative to site, the year-by-site interaction explained less of the

variation in all cases (6% as much for green spot number to 72% as much for carotenoid colour relative area) except for melanic colour relative area, where neither effect was strong. The strength of the year effect and the overall trend through time toward increasing total colour is almost certainly the result of improvements in photographic methods and equipment. In particular, we gradually transitioned from point and shoot cameras with onboard flashes to digital SLRs with commander ring flash set-ups. We also transitioned from rudimentary backgrounds and automatic camera settings (which are sensitive to changing conditions) to standardized backgrounds and full-manual control of settings (e.g. shutter speed, aperture, white balance, and ISO).

We now consider colour variation among specific sites, as well as temporal consistency in that variation. In this assessment, we will often refer to predation regime. Our intent in making this reference is not to imply a test for how predation regime influences colour because the present study was not designed as another test of this well-established association (Endler, 1980; Rodd & Reznick, 1997; Millar *et al.*, 2006; Karim *et al.*, 2007; Martin & Johnsen, 2007; Kemp *et al.*, 2009; Weese *et al.*, 2010). Instead, our references to predation regime draw on this prior knowledge to aid post-hoc interpretations of the causes of spatial variation in guppy colour.

Sites where guppies had the highest relative area of total colour were LP sites (M4, M16, P4, and P7), and this pattern was relatively consistent across all years (Fig. 1; see also Supporting information, Table S1). Specifically, adjusted means for these four sites were the highest among all sites within each of the six years, with only two minor exceptions (Fig. 1; see also

Table 2. Results from the univariate analyses of covariance with year, site, and their interaction as fixed effects; relative area (arcsine square root transformed) or spot number (square root transformed) for individual colour categories (carotenoid, structural, melanic, and green) as dependent variables; and body size (mm²; log transformed) as the covariate

	Carotenoid				Structural			
	<i>F</i>	<i>P</i>	Partial η^2	d.f.	<i>F</i>	<i>P</i>	Partial η^2	d.f.
Relative area								
Site	13.923	< 0.001	0.311	5658	14.085	< 0.001	0.294	5658
Year	5.814	< 0.001	0.091	5658	7.716	< 0.001	0.252	5658
Year-by-site	2.527	< 0.001	0.088	25 658	3.703	< 0.001	0.123	25 658
Body area	2.449	0.118	0.000	1658	46.801	< 0.001	0.087	1658
Spot number								
Site	12.982	< 0.001	0.100	5658	63.313	< 0.001	0.375	5658
Year	0.696	0.627	0.099	5658	15.595	< 0.001	0.119	5658
Year-by-site	3.2717	< 0.001	0.087	25 658	3.272	< 0.001	0.111	25 658
Body area	51.835	< 0.001	0.003	1658	134.399	< 0.001	0.062	1658
	Melanic				Green			
	<i>F</i>	<i>P</i>	Partial η^2	d.f.	<i>F</i>	<i>P</i>	Partial η^2	d.f.
Relative area								
Site	1.869	0.098	0.059	5658	10.511	< 0.001	0.223	5658
Year	8.750	< 0.001	0.276	5658	6.254	< 0.001	0.225	5658
Year-by-site	2.418	< 0.001	0.084	25 658	3.482	< 0.001	0.117	25 658
Body area	0.119	0.730	0.001	1658	3.849	0.0502	0.015	1658
Spot number								
Site	2.695	0.020	0.093	5658	3.523	0.004	0.081	5658
Year	5.853	< 0.001	0.076	5658	4.359	< 0.001	0.117	5658
Year-by-site	1.267	0.174	0.046	25 658	2.172	< 0.001	0.076	25 658
Body area	0.178	0.674	0.000	1658	3.825	0.051	0.009	1658

Statistical significance ($P < 0.05$) is indicated in bold.

Supporting information, Table S1). Interestingly, high total colour was achieved very differently by guppies from one of these LP sites (M16) than by guppies from the other LP sites, a pattern again consistent across years (Fig. 1; see also Supporting information, Tables S1, S2). In particular, and with only minor exceptions, guppies from the M4, P4, and P7 sites exhibited higher carotenoid colour and lower structural colour than did guppies from the M16 site (Figs 1, 2, 3; see also Supporting information, Tables S1, S3, S4). Conversely, guppies from the M16 site exhibited lower levels of carotenoid colour, higher levels of structural colour, and higher levels of green (Figs 1, 2, 3; see also Supporting information, Tables S1, S3, S4). Overall, spatial variation in colour was relatively consistent through time, both in its generality (guppies from the four LP sites always had the highest total colour) and in its anomalies (guppies from one LP site attained high colour in a very different way than guppies from the other three LP sites). Consideration of river drainage (Marianne or

Paria) as a potential effect does not significantly alter our results or interpretations of the data (see Supporting information, Tables S5, S6)

Results for spot number were similar to those described above for relative colour area, and so they are only briefly summarized here (for details, see Supporting information, Fig. S2, Table S2, S3, S4). In particular, strong spatial patterns were again evident and were generally consistent through time. As one example, the site where guppies had the most colour spots was M16 in all six years (see Supporting information, Fig. S2, Table S2). As another example, guppies from M7 and M14, both HP sites, generally had more structural colour spots than did guppies from three LP sites: M4, P4, and P7 (see Supporting information, Fig. S2, Tables S2, S3, S4).

Millar *et al.* (2006) reported a strong negative association between mean relative areas of orange and of blue among guppy populations from the Marianne and Paria rivers. We found the same association ($r = -0.881$; $P = 0.020$) in our dataset based on six of

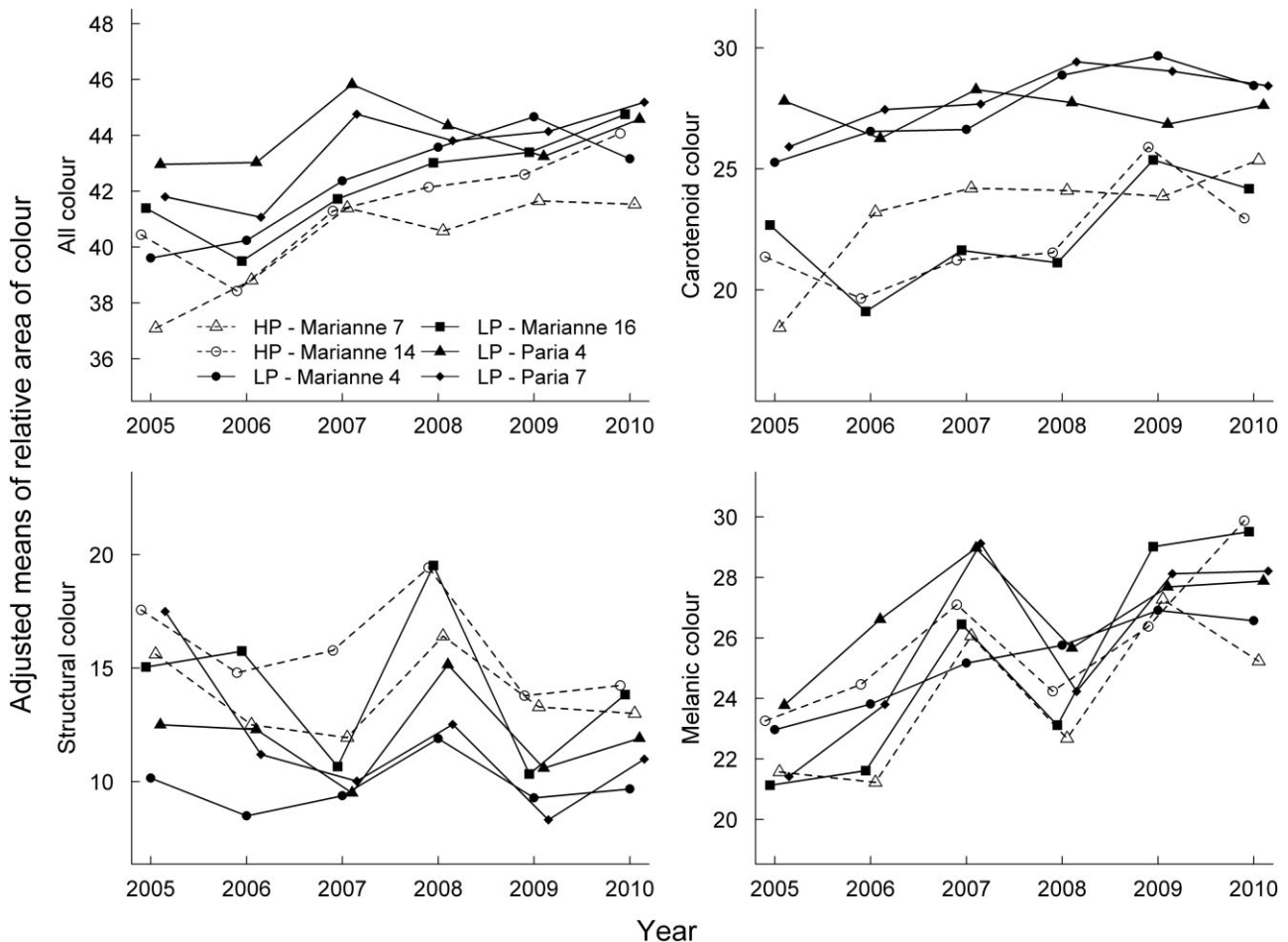


Figure 1. Adjusted means for the relative area of colour categories (all colour, carotenoid colour, structural colour, and melanic colour) by year and site. Data were arcsine square root transformed. Error bars are not presented for clarity when interpreting the graph but are reported in the Supporting information (Table S1). High-predation (HP) sites are indicated with open symbols and dashed lines; low-predation (LP) sites are indicated with filled symbols and solid lines.

these sites when all years were combined (Fig. 2). This negative relationship was also present across all six years, although it was statistically significant in only three years (see Supporting information, Table S7). Perhaps the most interesting part of this analysis is the position of M16 in relation to the other sites. Although an LP site, M16 has colour patterns more similar to guppies from an HP site than to those for guppies from other LP sites (Fig. 2). Again, this distinction for M16 guppies was consistent across all years.

DISCUSSION

Some temporal variation was evident in the present study. The main effect of year is likely attributable to improved photographic methods and equipment (see Results) and so is unlikely to be biologically relevant. The interaction between site and year was also often

significant in both multivariate and univariate models, and might well reflect temporal variation in selection. Currently, we do not have a specific causal explanation for this variation because we did not measure environmental variables at each site over all 6 years. Thus, it might be worthwhile to search for potential causes of this temporal variation in future work. However, we do not further discuss this temporal variation for the above reasons and because (as explained below) its effect was minor in relation to spatial variation.

Three main observations highlight the predominant effect of spatial variation over temporal variation in guppy colour. First, guppies from three populations (M4, P4, and P7) in LP environments consistently had the highest total colour, the highest carotenoid colour, and the lowest structural colour (Fig. 1; see also Supporting information, Fig. S2). Second, guppies from the fourth LP population (M16) also had high

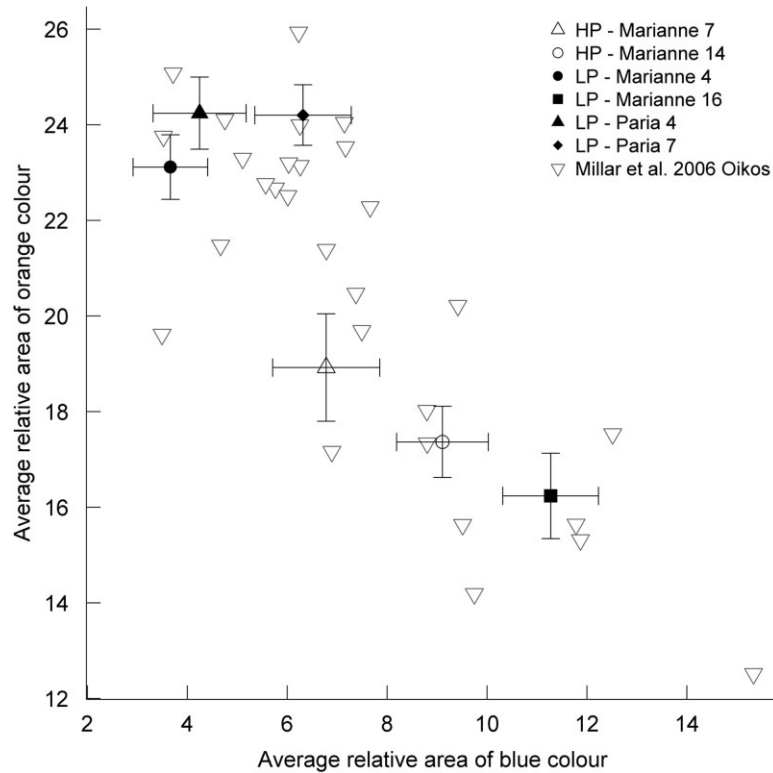


Figure 2. The mean relative area of orange and blue spots is inversely related among sites in the Marianne and Paria Rivers. Error bars denote 95% confidence intervals for all years combined. Grey triangles are data from a previous study of 29 sites in the Marianne and Paria rivers (Millar *et al.*, 2006).

total colour but differed from the other three LP populations in consistently having low carotenoid colour, high structural colour, and high green (Fig. 1; see also Supporting information, Fig. S2), which is also consistent with previous work (Schwartz & Hendry, 2010; Weese *et al.*, 2010). Third, the previously-described negative association among populations for relative orange versus blue colour on male guppies (Millar *et al.*, 2006) was essentially identical and consistent across years in our new samples (Fig. 2). These consistent spatial patterns likely reflect genetically-based adaptive responses to spatial variation in selection (details below). The alternatives of drift and plasticity appear less likely because (1) the populations we sampled are large enough that genetic drift is likely minor and (2) variation in the size and number of colour spots is strongly genetically based (Houde, 1992; Brooks & Endler, 2001; Karino & Haijima, 2001; Hughes *et al.*, 2005; Tripathi *et al.*, 2009; Gordon *et al.*, 2012). Of course, it is important to recognize that a field study such as ours cannot disentangle genetic effects from any plastic effects on male guppy colour patterning. Furthermore, more plastic components of guppy colour, such as carotenoid deposition, brightness, and

chroma, might vary more temporally and would be useful to study in future work.

Biological explanations for the first observation (three LP sites had high colour, high carotenoid, and low structural colour) and the third observation (a negative relationship between carotenoid and structural colours) follow naturally from previous work on guppy colour. In particular, high orange in guppies from LP sites is considered to reflect strong female preferences for orange (Kodric-Brown, 1985; Long & Houde, 1989; Houde & Endler, 1990; Endler & Houde, 1995; Grether, 2000; Brooks & Endler, 2001; Pilastro *et al.*, 2004) coupled with an absence of major predators that select against orange (Endler, 1978; Endler, 1991; Godin & McDonough, 2003; Millar & Hendry, 2012). In addition, high blue but low orange in guppies from high-predation (HP) sites (here M7 and M14) is considered to reflect female preference for colourful (including blue) males (Kodric-Brown, 1985; Houde, 1997) and the presence of major predators that select against orange but are less sensitive to blue (Endler, 1978; Archer & Lythgoe, 1990; Kröger, Bowmaker & Wagner, 1999; Kemp *et al.*, 2008).

A biological explanation for the second observation (the M16 LP site had high colour but in a different

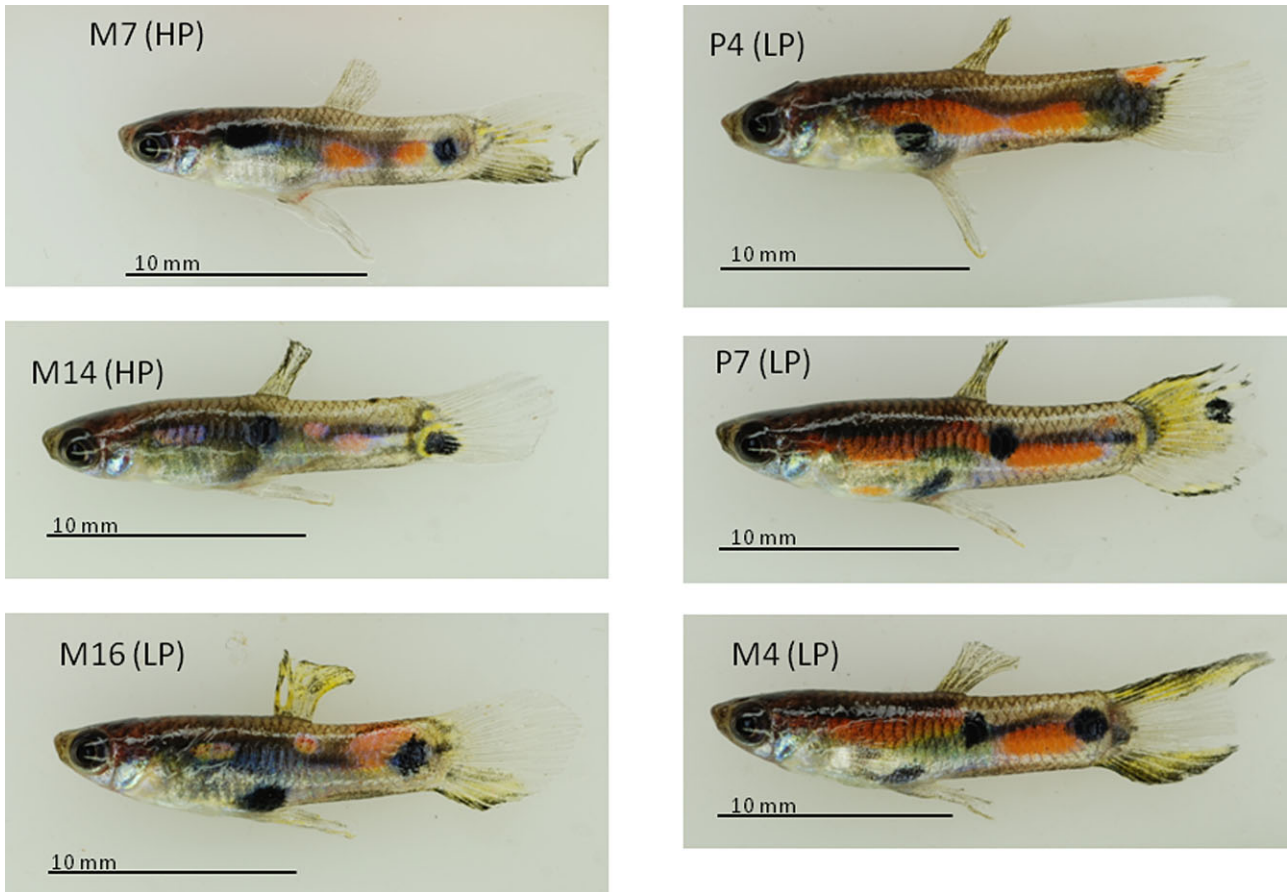


Figure 3. Representative photographs of male guppy colour patterning. All photographs are from 2009, although the colour on each fish shown in the photograph is representative of the overall mean for a given site. Photographs on the left represent sites where males have less carotenoid colour and photographs on the right represent sites where males have more carotenoid coloration. Black scale bars = 10 mm. Photographs are not scaled with respect to each other. HP, high-predation; LP, low-predation.

way from other LP sites: more structural colour than carotenoid colour) does not emerge so easily from previous work and so deserves further consideration. The fact that M16 guppies were anomalous across all 6 years suggests a consistently atypical local selective pressure. One possibility is a unique predator fauna, although we have not noted any predator differences between M16 and the other LP sites during 10 years of visits. Another possibility is the more open canopy at M16 in comparison with the other LP sites (McKellar *et al.*, 2009; Schwartz & Hendry, 2010); however, we would expect the resulting higher productivity to favour increased (not decreased) carotenoid colour (Grether *et al.*, 2001b). A third possibility is limited genetic variation in comparison with other LP sites. This is unlikely because M16 has comparable genetic diversity to other Marianne LP sites (Crispo *et al.*, 2006), and colour variation is not lower among individuals within M16 compared to other LP

sites (see Supporting information, Tables S1, S2). A final possibility is that anomalous patterns of sexual selection at M16 have favoured an atypical male colour pattern. This last explanation is attractive given (1) a lack of support for the above alternatives; (2) evidence that females from different populations show different preferences for male colour (Endler & Houde, 1995; Brooks & Endler, 2001); and (3) evidence for among-population correlations between female preference and male colour (Houde & Endler, 1990; Schwartz & Hendry, 2007). However, the reasons why females from this site might prefer an anomalous colour pattern are not known, although the initial impetus could be female preferences for novel males (Hughes *et al.*, 2013). Overall, temporal consistency of the anomalous colour pattern at this site suggests the value of more formally exploring these, as well as other, potential explanations. Of particular value would be formal selection estimates

across years at M16 and other sites of particular interest.

SPACE VERSUS TIME

We found that, although some temporal variation was present, it did not strongly modify spatial variation in male colour, and this result might be considered surprising in light of evidence for temporal variation in biotic and abiotic factors that influence selection (see Introduction). We suggest that temporal variation in selection is indeed present but is overpowered by spatial variation, at least on the spatiotemporal scales investigated in the present study. In particular, (1) LP populations never have dangerous predatory fishes, whereas HP populations often do (Endler, 1980); (2) canopy cover differs among sites but is reasonably consistent through time at a given site in the absence of human disturbances (Schwartz & Hendry, 2010); (3) parasitism varies much more spatially than temporally (Fraser, Ramnarine & Neff, 2010; Gotanda *et al.*, 2013); and (4) factors (e.g. sex ratio) that likely influence sexual selection also appear to vary more spatially than temporally (McKellar *et al.*, 2009). All of these observations support our present results in suggesting that the adaptive consequences of temporal variation in selection are weaker than the adaptive consequences of spatial variation in selection.

An alternative is that temporal variation in selection is strong but, for various reasons, the response or consequence of this selective variation does not cause notable phenotypic changes on the time scale we considered. First, colour might evolve so slowly that adaptive phenotypes are reasonably stable despite temporal fluctuations in selection. This explanation is unlikely to be sufficient given the high heritability of colour (Houde, 1992; Brooks & Endler, 2001; Karino & Haijima, 2001; Hughes *et al.*, 2005; Tripathi *et al.*, 2009; Gordon *et al.*, 2012) and a number of studies showing rapid colour changes in guppies introduced to new environments (Endler, 1980; Kemp *et al.*, 2008), although this is not always the case (Karim *et al.*, 2007; Kemp *et al.*, 2009). Second, temporal variation in selection might be swamped, or at least dampened, by gene flow among sites. This explanation also appears unlikely given our documentation of low gene flow among these sites (Crispo *et al.*, 2006; Weese *et al.*, 2011) and the strong spatial variation that would be likewise opposed by high gene flow. Additionally arguing against both of these alternatives, the spatial variation in our study is large and sufficiently stable to suggest that the populations are reasonably well adapted to alternative fitness peaks. For all of these reasons, we favour the explanation that, when considering the variation in an adaptive

trait under selection, the selective factors influencing male guppy colour, and therefore selection itself, vary more in space than in time.

Our conclusion that temporal variation in selection contributes less than spatial variation in selection to colour pattern variation might be used to support the perspective that directional selection is relatively consistent through time (Morrissey & Hadfield, 2012). However, we caution against too strong an interpretation in this direction owing to the properties of the guppy system. In particular, guppies have long been known to exhibit very strong spatial variation in selection and traits, and have been a preferred study system for this reason (Endler, 1978; Endler, 1980; Magurran *et al.*, 1992; Carvalho *et al.*, 1996; Kelly, Godin & Wright, 1999; Millar *et al.*, 2006; Kemp *et al.*, 2009; Millar & Hendry, 2012). Indeed, the spatial variation in male guppy colour is so strong and clear that no study of non-manipulated populations had yet temporally replicated its samples. Moreover, we specifically selected populations for our long-term study that were known from previous work (Millar *et al.*, 2006) to occupy different selective environments and to differ in their colour patterns. In short, the guppy system in general, and our study sites in particular, might represent a situation in which the importance of spatial variation in selection is exceptionally (perhaps atypically) strong in relation to temporal variation in selection.

CONCLUSION AND APPLICATIONS

We found that temporal variation, although present, was too low to modify interpretations regarding spatial variation in an adaptive trait: male guppy colour. We suggest that this result reflects selection that varies much more in space than in time, at least for the populations and time frame examined in the present study. Although these observations might be taken to support the idea that selection is reasonably consistent through time, our study system might be exceptional in this regard. We suggest that analyzing spatiotemporal variation in adaptive traits provides a complementary basis for considering the importance of temporal variation in selection. In doing so, it is critical to note that the adaptive evolution of traits is not necessarily expected to match selection coefficients estimated in natural populations (Merilä *et al.*, 2001; Haller & Hendry, 2014). We are confident that our results do reflect the action of selection owing to the specific properties of our study system as discussed above. Similar inferences for other systems would require similar background evidence that traits are genetically based and responsive to natural selection, and that genetic drift and gene flow are not too strong.

Analyses such as those conducted in the present study require data on genetically-based, adaptive phenotypes in multiple populations over multiple years/generations, and so it is worth considering whether such data are likely to be commonly available. A first important point is that any system with data to address spatiotemporal variation in selection automatically has the data to examine spatiotemporal variation in the traits themselves. Indeed, such datasets are optimal because they allow consideration of the extent to which spatiotemporal variation in evolution and selection line up with each other, as they might or might not depending on the situation (see Introduction). A second important point is that the examination of trait variation will be possible for many additional data sets because it does not require individual-based data (population means are sufficient) or fitness estimates. Examples of such data sets include body size and shape in salmonids (Carlson & Quinn, 2007), colour patterns in *Cepaea* snails (Silvertown *et al.*, 2011), pelvic structures in threespine stickleback (Klepaker *et al.*, 2012), gall size in *Eurosta* flies (Weis, Abrahamson & Andersen, 1992), and morphological traits in deer mice (Pergams & Ashley, 1999). We note that, for the same reasons given above, it would also be extremely useful to obtain estimates of selection for such systems. In short, we are confident that many data sets exist for which spatiotemporal variation in adaptive traits can be examined, but we again caution that interpretations always should be conditioned by information on the genetic basis and adaptive significance of the studied traits.

We can see several useful extensions to the approach advocated here. First, we studied a trait that was genetically-based, but it also would be interesting to consider spatiotemporal variation in very plastic traits. For example, we might predict that plasticity would lead to greater temporal variation because plastic traits are presumably able to respond more quickly to a local environmental change (Stearns, 1989; Lande, 2009; Pfennig *et al.*, 2010). In our system, this might be true if we had quantified some of the more plastic components of colour such as carotenoid deposition, brightness, and chroma. Second, we examined populations for which genetic drift was likely unimportant, yet it would also be interesting to test whether very small populations show greater temporal variation in the spatial patterns of a phenotypic trait. Third, we emphasized phenotypic variation in quantitative traits, but similar analyses could be performed for frequencies of phenotypic or genetic polymorphisms. Overall, we suggest that analyses of spatiotemporal variation in adaptive traits complement (without replacing) analyses of spatiotemporal variation in selection coefficients.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Map of the Marianne and Paria rivers on the northern slope of the Trinidadian mountain range.

Figure S2. Spot numbers of colour category by year and site

Table S1. Mean \pm SE at each site in each year for relative areas (arcsine square root transformed) of each colour category (carotenoid, structural, melanic, and green). Also shown is the relative area of all colour categories combined (total).

Table S2. Mean \pm SE at each site in each year for number of coloured spots of each colour category (carotenoid, structural, melanic, and green). Also shown is the mean number of spots of all colour categories combined (total).

Table S3. Adjusted *P*-values for post-hoc Tukey's honestly significant difference test results on pairwise comparisons between sites for carotenoid coloration. Top right is for relative area and lower left is for spot number. Statistical significance ($P < 0.05$) is indicated in bold.

Table S4. Adjusted *P*-values for post-hoc Tukey's honestly significant difference test results on pairwise comparisons between sites for structural coloration. Top right is for relative area and lower left is for spot number. Statistical significance ($P < 0.05$) is indicated in bold.

Table S5. Results from multivariate analysis of covariance using Wilk's lambda test statistic with site fitted before year as fixed effects and body size (mm^2 ; log transformed) as the covariate for sites within the Marianne river. Estimates of variance were calculated as multivariate partial η^2 also utilizing Wilk's lambda. The dependent variables were relative area (arcsine square root transformed) and spot number (square root transformed) for all colour categories (carotenoid colour, structural colour, melanic colour, and green). Statistical significance ($P < 0.05$) is indicated in bold.

Table S6. Results from multivariate analysis of covariance using Wilk's lambda test statistic with site fitted before year as fixed effects and body size (mm^2 , log transformed) as the covariate for sites within the Paria river. Estimates of variance were calculated as multivariate partial η^2 also utilizing Wilk's lambda. The dependent variables were relative area (arcsine square root transformed) and spot number (square root transformed) for

all colour categories (carotenoid colour, structural colour, melanic colour, and green). Statistical significance ($P < 0.05$) is indicated in bold.

Table S7. Pearson-product moment correlation test results comparing the relative area of blue and orange colour for individual years. Statistical significance ($P < 0.05$) is indicated in bold.

Table S8. Results from multivariate analysis of covariance (MANCOVA) using Wilk's lambda test statistic with year as a randomized block, site as a fixed effect, the year-by-site interaction, and body size (mm^2 ; log transformed) as the covariate. All MANCOVAs were carried out in the R working environment utilizing the car package. The dependent variables were relative area (arcsine square root transformed) and spot number (square root transformed) for all colour categories (carotenoid, structural, melanic, and green). Statistical significance ($P < 0.05$) is indicated in bold.

ARCHIVED DATA

Data deposited at Dryad (Gotanda & Hendry, 2014).