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A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*)

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ABSTRACT

Question: Do male traits and female preferences co-evolve in response to divergent natural selection?

Organisms: Six Trinidadian guppy (*Poecilia reticulata*) populations adapted to high- or low-predation environments in three separate drainages.

Methods: Measurement of colour patterns on wild-caught and lab-reared males. 'No-choice' mating experiments to quantify female preference functions for male traits. Comparisons of male colour and female preference functions between predation environments.

Predictions: If divergent natural selection drives parallel co-evolution, both male traits and female preferences should be similar for populations in similar environments but different for populations in different environments.

Conclusions: Male traits have broadly diverged in parallel between predation environments, leading to larger body size and increased colour in low-predation sites. Female preferences also appear to be diverging because females discriminate against colourful males in high-predation sites but not in low-predation sites. Despite this general pattern, deviations from parallel co-evolution were also present, suggesting a substantial role for other selective agents.

Keywords: adaptation, divergent selection, ecological speciation, guppy, mate choice, predation, preference function, sexual selection.

INTRODUCTION

Parallel evolution occurs when closely related, but independent, lineages show similar phenotypic adaptation to similar ecological environments (Williams, 1972; Jones *et al.*, 1992; Schluter, 1996). As such, parallel evolution provides strong evidence for the role of natural selection in driving population differentiation (Schluter, 2000). A key to revealing parallel evolution is the use of multiple populations that have independently colonized different ecological environments. Specifically, parallel evolution predicts that phenotypes will be correlated with ecological environments, rather than geographical or historical effects (Schluter, 2000; Langerhans and DeWitt, 2004).

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Parallel evolution may be particularly interesting in the case of secondary sexual traits, because these respond to both natural and sexual selection. In particular, these traits often evolve for maximum detectability to potential mates or rivals (i.e. sexual selection), a conspicuousness that can also make them direct targets of natural selection (Fisher, 1930; Lande, 1981; Kirkpatrick, 1987; Skroblin and Blows, 2006). The optimal phenotype of secondary sexual traits may therefore reflect a balance between the relative influences of natural and sexual selection (Endler, 1992). When natural selection differs among populations, so too will the balance with sexual selection, and therefore the optimal phenotype. Almost all studies examining the parallel evolution of secondary sexual traits proceed in this vein by focusing on the role of varying natural selection (e.g. Slabbekoorn and Smith, 2002; McKinnon *et al.*, 2004; Stoks *et al.*, 2005). Here we emphasize the additional role of varying sexual selection among environments (e.g. Svensson *et al.*, 2006), especially in the context of female preference.

On the one hand, male traits and female preferences may co-evolve because both are subject to divergent natural selection (Kirkpatrick, 1996; Fuller *et al.*, 2005), or because female preference evolution is a by-product of divergence in male traits (Lande, 1982; Day, 2000). In this case, both natural and sexual selection may act in concert, rather than in opposition, and thereby synergistically drive divergence among environments. On the other hand, male traits and female preferences may not co-evolve closely, perhaps because female preferences evolve in response to different selective pressures. As one example, the benefits of mating with males carrying a particular trait may be relatively consistent across environments, such as in the case of good genes/sexy sons, or due to pre-existing biases in the female sensory system (Ryan and Rand, 1993; Schluter and Price, 1993; Endler and Basolo, 1998). In these cases, natural and sexual selection may sometimes act in opposition to each other, and thereby constrain divergence among environments. The extent to which male traits and female preferences co-evolve and are influenced by environmental gradients will have major implications for: (1) the origin and maintenance of differentiation among populations, and (2) the ability of males to reach the optimal phenotype from the perspective of natural selection. Our goal is to test for these possibilities in natural populations by looking for signatures of parallel evolution in response to both natural and sexual selection.

The parallel evolution of male traits is assessed simply by comparing trait values across replicate populations in different ecological environments (Schluter, 2000). A similar assessment for female preferences can be made by comparing the shape of population-level preference functions – that is, the relationship between male trait values and female preferences (Rundle *et al.*, 2005; Schwartz and Hendry, 2006). If divergent natural selection predictably drives female preferences, then (1) preference functions should be more similar among populations from similar environments than among populations from different environments, and (2) the direction of divergence in preference functions should match the direction of divergence in male traits. If divergent natural selection does not predictably drive female preference evolution, then we might expect different patterns of divergence. Here we ask how divergent natural selection influences the co-evolution of sexual signals and female preferences for those signals in Trinidadian guppies (*Poecilia reticulata*) from three lineages that independently colonized divergent predation environments.

The guppy system

Guppies offer excellent opportunities to study parallel evolution in response to predation (Endler, 1980, 1995; Reznick *et al.*, 1996; Magurran, 2005). Predation intensity generally varies along the upstream/downstream axis of rivers, with sharp changes occurring across waterfalls that prevent upstream colonization by predatory fishes (Haskins *et al.*, 1961). As a result, headwaters and tributaries are generally low-predation environments, whereas downstream sections are generally high-predation environments. Owing to this divergent natural selection, high- and low-predation populations within a river show adaptive divergence in behaviour, life history, and male colour (Seghers, 1974; Endler, 1978, 1980; Reznick and Endler, 1982; Magurran, 2005). Of particular relevance to the present study, males in low-predation environments are more colourful than males in high-predation environments (Endler, 1980; Millar *et al.*, 2006). This adaptive difference is thought to arise because, although females generally prefer more colourful males (Houde, 1987; Endler and Houde, 1995; Brooks and Endler, 2001), these males have lower survival in high-predation environments than in low-predation environments. Adaptive divergence between high- and low-predation environments has occurred independently in multiple drainages, and therefore represents parallel evolution (Endler, 1995; Reznick *et al.*, 1996; Magurran, 2005).

Male signalling traits therefore diverge predictably in response to the difference in the strength of natural selection acting on them, but what about female preferences? In general, preference functions should evolve as a compromise between the benefits and costs of mating with males carrying preferred traits (Schluter and Price, 1993; Jennions and Petrie, 1997; Widemo and Saether, 1999). Because predation targets colourful males, the costs of carrying this trait can be high in high-predation environments (Endler, 1980). It is also possible that high-predation environments may select against females with preferences for colourful males. First, males with large amounts of orange will be rarely encountered in high-predation populations, potentially increasing search times. Second, proximity to a colourful male may increase a female's risk of predation (Pocklington and Dill, 1995; Gong and Gibson, 1996). Third, females that mate with colourful males will produce colourful male offspring that may have lower survival owing to predation. If no greater benefit is gained by mating with a colourful male, then female preference functions for male colour may evolve to match the male trait value determined by the natural selection optimum.

Previous work suggests the potential for adaptive genetic variation in female preferences (Houde and Endler, 1990; Endler and Houde, 1995; Brooks and Endler, 2001; Kodric-Brown and Nicoletto, 2001). In particular, female preferences for increased orange are weaker in populations where males are less orange, suggesting the co-evolution of traits and preferences (Houde and Endler, 1990; Rodd *et al.*, 2002). Furthermore, although some research has suggested that females in high-predation populations have weaker preferences for colour than do females in low-predation populations (Breden and Stoner, 1987; Stoner and Breden, 1988; Houde and Endler, 1990; Endler and Houde, 1995), the role of divergent predation pressure in driving this apparent co-evolution has not yet been examined. Here, we formally test for the parallel evolution of both male traits and female preferences between high- and low-predation environments.

METHODS

Populations

Adult guppies were collected in March 2003 and March 2004 from paired high-predation (downstream) and low-predation (upstream) localities in three separate drainages along Trinidad's Northern Range mountains: (1) the Aripo River on the south slope in the western Orupuche drainage, (2) the Quare River on the south slope in the eastern Caroni drainage, and (3) the Yarra River on the north slope draining into the Caribbean Sea (see

Table 1. Locations of the study populations in Trinidad's Northern range mountains

Site	Predation risk	Grid reference	Drainage
Upper Aripo tributary (Naranjo river)	Low	PS 933 818	Caroni
Lower Aripo	High	PS 780 940	Caroni
Upper Quare tributary	Low	PS 810 970	Orupuche
Lower Quare	High	PS 792 975	Orupuche
Upper Yarra tributary (Limon river)	Low	PS 834 876	Northern
Lower Yarra	High	PS 802 940	Northern

Note: Grid references are from the Trinidad National Grid System 1 : 25,000 map series.

Table 1 for grid references). These rivers were chosen because they allow an appropriate test for parallel evolution. First, genetic data have revealed that guppies in the Orupuche drainage have been isolated from those in the other two drainages for more than 500,000 years (Carvalho *et al.*, 1991; Fajen and Breden, 1992). Second, guppies in the Yarra River on the north slope have been long isolated from those on the south slope, owing to the precipitous mountain range that separates them. Third, high-predation and low-predation environments on the north slope are characterized by different suites of predators than are those on the south slope. This last feature allows a test for the evolutionary importance of predation in general, rather than a particular type of predator (Reznick *et al.*, 1996). The two sampled populations within each river were separated by at least one barrier waterfall, and were far enough apart for gene flow from the opposing predation environment to be low.

Fifteen to twenty pregnant females from each of the six populations were transported to our laboratory at McGill University. We also collected 20 males from each site so as to determine natural variation in colour. These males were anaesthetized with tricaine methanosulphate (MS-222), placed on a standard grid-ruled background, illuminated with full-spectrum fluorescent lights, and photographed with a digital camera (Nikon Coolpix 995). Colour and body size were then measured from the digital photographs using Scion Image Software (www.scioncorp.com). Each colour spot was first classified into one of three main categories (orange/red, black, and structural colour, the latter including blues, violets, and greens). The entire body and each spot was then outlined and the size (mm^2) of the enclosed area determined. [For details on these methods see Millar *et al.* (2006).] The total area of a given colour on a given male may be influenced by his overall body size, and so all analyses are based on the relative area of each colour on each male (total area of a colour divided by total body area). The absolute and relative size of the tail and its colour spots were measured separately, but in the same way.

Laboratory maintenance and rearing

Wild-caught females were individually isolated in 9.5-litre (30.8 × 13.7 × 21 cm) glass aquaria, each containing an airstone and 'Java Moss'. The isolated females then gave birth on a regular schedule without the need for further matings – because guppies mate multiply in the wild, are internally fertilized, bear live young, and can store sperm for up to 8 months (Liley, 1966; Becher and Magurran, 2004). Newly born offspring were removed from their mother's aquarium and held in family-specific 20.8-litre (40.9 × 20.6 × 25.7 cm) aquaria, each with a sponge filter and with gravel covering the bottom. These offspring tanks were checked daily and males were removed before they completed sexual maturation. This procedure ensured that the females were virgins before a mating trial, and therefore receptive to mating (Baerends, 1955; Liley, 1966). Juveniles were fed twice daily a diet of live brine shrimp, whereas adults were fed once daily an alternating diet of liver paste and crushed flakes.

Mate choice trials

The experiments took place in 20.8-litre tanks with three sides covered by brown paper and the bottom covered by a single layer of mixed-colour gravel. The tanks were illuminated by an overhead full-spectrum fluorescent bulb (Vita-Lite 40 W, Duro-Test Canada), and were isolated and screened from all other external disturbances. The full spectrum lighting provided an irradiance range from at least 350 to 700 nm, which we verified using a spectroradiometer (SD 2000, Ocean Optics). We reduced the intensity of light by covering the experimental tank with layers of cheesecloth. A wide spectral range at reduced intensity is important because all of these wavelengths can be present in the natural environment at dawn and dusk, when courtship is often at its highest (Endler, 1987; Gamble *et al.*, 2003).

The mate choice trials involved a total of 120 guppies (10 male/female pairs for each of the six populations), with each fish used only once. All experimental fish were the laboratory-reared, first-generation offspring of wild-caught females, and all pairs were from different families to avoid potential inbreeding effects on behaviour. Several study designs are possible for mate-choice experiments (Houde, 1997). We employed a 'no-choice' design (Houde, 1997), which eliminated social interactions that potentially affect male and female behaviour (e.g. Dugatkin and Godin, 1992). Although the social environment of courtship may ultimately affect mate choice in the wild, we are here concerned with *intrinsic* female preferences. It was therefore appropriate to eliminate social interactions.

For each of the 60 trials, a male was first placed alone into the experimental tank and allowed to acclimate for a minimum of one hour. A female from the same population was then added to the tank and their interactions were video-taped (Canon XL1-S). The duration of each trial was either 30 min (if no copulation occurred) or 10 min beyond the first successful copulation, whichever period ended first. Successful copulation occurred in 51% of the trials, and was higher for high-predation populations (62%) than for low-predation populations (39%; t-test: $P = 0.03$). These intermediate mating rates, along with the overall variation in female responses (see Results), show that naïve females are not indiscriminately accepting of all males. Variation in response of virgin females in our experiment therefore should be useful in assessing variation in preference functions.

Measuring female preference

The strength of a female's preference for her test male was determined as the intensity of her responses to his displays. Typical guppy courtship involves a male orienting in front of a female, arching his body, and vibrating quickly in what is known as a 'sigmoid display' (Baerends *et al.*, 1955; Liley, 1966; Houde, 1997). Males will usually perform several displays in front of a female before she may consent to copulation. Females indicate their acceptance of a male by (1) orienting towards the male, (2) approaching in a 'glide', and (3) circling around the male until (4) he is able to attempt copulation. (5) Successful fertilization is indicated by male 'jerking', cessation of courtship, and close guarding of the female for up to 10 min. Trials were discarded if males displayed less than five times or if females did not show a single ‘glide’ response. This ensured that only receptive and potentially responsive females were used in the experiments.

We categorized the intensity of each female’s response to each male display using the above 1–5 scale (after Houde, 1997). The sum of all scores for a female within a trial gives her total response to the male (TR). Individual males vary substantially in the number of displays, and so TR reflects variation in both male behaviour (number of displays) and female behaviour (response to each display). To standardize for male behaviour, we divided the observed TR for each female by the maximum response she could have shown to that male over the same number of displays. This maximum response (MR) would be a score of ‘4’ for each display prior to copulation plus a ‘5’ for the display that led to copulation: $MR = ([\text{number of displays} - 1] \times 4) + 5$. We refer to this standardized female response as the ‘fractional intensity of response’ ($FIR = TR/MR$). This index differs only slightly from Houde’s (1997) ‘fraction response’ (FR), where only scores of ‘2’ or higher are divided by the total number of displays. We chose FIR in preference to FR because we feel the former captures more information about a female’s willingness to mate. Regardless, FIR and FR are highly correlated ($r^2 = 0.78$) and yield very similar preference functions (results not shown).

Female preference functions can take a variety of shapes (Lande, 1981; Blows *et al.*, 2003; Bentsen *et al.*, 2006), with the simplest being a linear association between female response (y-axis) and male trait value (x-axis). We estimated linear preference functions for each population by regressing FIR (arcsine square-root transformed) on male traits that may influence female choice (Endler and Houde, 1995; Brooks and Endler, 2001). These traits were quantified for each experimental male by anaesthetizing (MS-222) him immediately after the trial, and then photographing him (Nikon coolpix F995) on a standard grid under a full-spectrum light. Colour and size were then measured following the protocol outlined above for the wild-caught males. Traits that did not conform to normality assumptions were transformed ($\log_{10}$ for absolute values and arcsine square root for relative values).

Statistical analysis

Variation in male traits was analysed as a crossed design mixed analysis of variance (ANOVA) with predation regime as a fixed effect and river as a random effect (SAS, version 8). Parallel evolution would be indicated by a significant effect of predation environment (i.e. high or low). A significant interaction, however, would indicate that the response to predation varied between rivers. Because we sometimes found such interactions (see Results), we also compared trait means between predation environments within each river (single-factor ANOVA). A separate analysis for each male trait might inflate the Type II error rate if any single significant test was interpreted as supporting the prediction. Bonferroni corrections are increasingly recognized as inappropriate (e.g. Moran, 2003; Garcia, 2004), and so we used the binomial likelihood function (Zar, 1999, pp. 518–522) to determine whether the number of significant tests was greater than expected by chance.

Variation in female preference functions (FIR as the response variable) was analysed by analysis of covariance (ANCOVA; SAS, version 8). A separate ANCOVA was run for each male trait, always using models that included predation regime as a fixed factor, river as a random factor, male trait value as a covariate, and all possible interactions. Our test for parallel evolution here began by examining the three-way interaction between predation, river, and male trait value. If significant, this would suggest population-specific differences that were not consistently associated with predation regime or river. A non-significant three-way interaction, however, would then enable a test for parallel evolution between predation regimes. After removing the three-way interaction term from the model, parallel evolution is revealed as a significant two-way interaction between predation and the male trait, indicating that the slopes of preference functions are different between predation environments, but not within them, in all three rivers. The binomial likelihood function was once again used to correct for potential errors from multiple univariate analyses. However, we were also specifically interested in variations in preference for particular traits previously found to be important (orange, black, tail size), and so these tests are also considered independent tests of separate hypotheses.

For visual depiction of preference functions, we used simple linear regressions between each trait and female preference within each population. We also used cubic splines (Schluter, 1988) to look for obvious non-linearities in the shape of preference functions. In agreement with previous work (Brooks and Endler, 2001; Blows *et al.*, 2003), none of the preference functions revealed any obvious deviations from linearity. We therefore present only the linear results.

RESULTS

Male colour

Consistent with previous studies (Endler, 1978, 1980; Millar *et al.*, 2006), wild-caught males generally showed parallel divergence between predation regimes for most colour and body size measures (Fig. 1; Table 2). Some significant predation-by-river interactions, however, suggested that the degree of divergence between predation regimes differs among rivers. We therefore also used river-specific analyses of variance (Table 3; Fig. 1). In the Quare and the Yarra rivers, low-predation males had significantly more orange and more black than their high-predation counterparts, but did not differ in structural colours. In the Aripo river, low-predation males had significantly more black and structural colouration than their high-predation counterparts, but did not differ in orange.

In contrast to wild-caught males, laboratory-reared males showed less evidence of colour divergence between predation regimes (Fig. 1; Tables 2 and 3). In fact, the only consistent *significant* differences were for orange in the Yarra and black in the Aripo rivers. Some of this reduction of significance for laboratory-reared males may reflect lower statistical power ($n = 20$ for wild-caught males, $n = 10$ for laboratory-reared males), but even the *direction* of divergence was not the same for some colours (Fig. 1). These differences between wild-caught and laboratory-reared males suggest the interesting possibility that some of the divergence in male colour in the wild may reflect either selection within a generation or phenotypic plasticity. Conveniently, the lack of divergence among populations in the laboratory was of help, because it allowed us to quantify preference functions across roughly the same range of colour in each population.

Preference-function divergence

Before looking for differences among populations in *how* traits are used in mate choice, we first attempted to determine *which* traits are used in mate choice in each population. Stepwise multiple-regressions within populations revealed an overall lack of consensus as to the traits of interest in mating decisions among populations. In the Aripo and Yarra rivers,

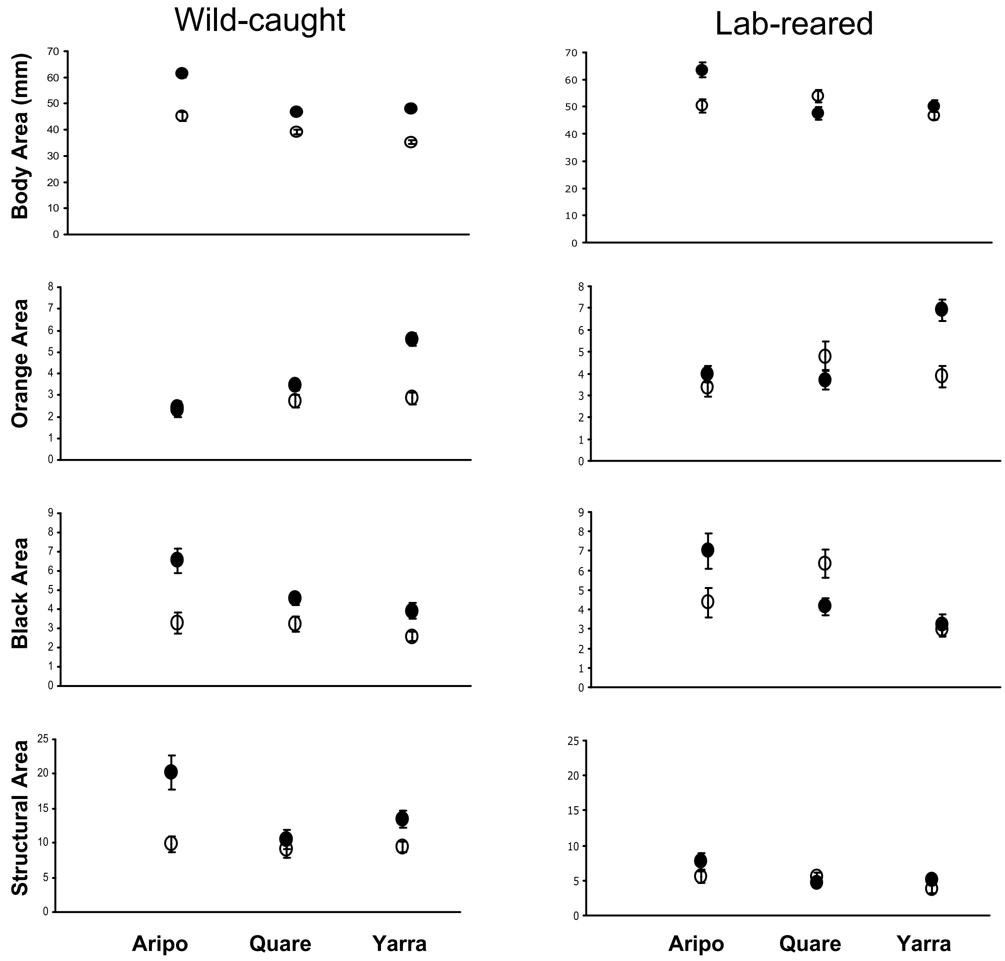


Fig. 1. Trait means (and standard errors) for wild-caught ($n = 20$ per population, left-hand panels) and laboratory-reared ($n = 10$ per population, right-hand panels) males for high-predation (open symbols) and low-predation (solid symbols) populations within each river.

small body size was the only trait significantly favoured by females from high-predation populations ($r^2 = 0.72$, $P = 0.004$ and $r^2 = 0.46$, $P = 0.05$, respectively), and no particular trait values were significantly favoured by females from low-predation populations (all $P > 0.05$). In the Quare river, more black spots was the only trait favoured by females from the low-predation population ($r^2 = 0.78$, $P = 0.001$), whereas fewer black spots was the only trait favoured by females from the high-predation population ($r^2 = 0.4$, $P = 0.04$).

When preference functions are considered for individual traits, again both the degree and direction of preference varied among populations (Tables 4 and 5; Fig. 2). Despite the above inconsistencies among populations, the *direction* of female preferences often differed consistently between predation environments, but not within them. These parallel aspects of divergence are indicated by (i) significant predation-by-trait interactions in the absence of significant three-way interactions (Table 4), and (ii) the signs of the slopes of population-

Table 2. Statistical analysis of male traits for wild-caught ($n = 20$) and laboratory-reared ($n = 10$) fish from paired high- and low-predation populations

	Wild-caught			Laboratory-reared		
Trait	Predation	River	Predation × River	Predation	River	Predation × River
Body area	143.21^{c}	51.94^{c}	5.86^{b}	3.4	7.19^{c}	8.89^{c}
Tail area	42.53^{c}	3.49^{a}	0.87	0.45	1.45	4.29^{a}
Orange area	23.94^{c}	18.94^{c}	10.34^{c}	4.16^{a}	6.03^{b}	8.58^{c}
No. of orange spots	8.47^{b}	10.26^{c}	1.05	0.20	0.17	0.79
Black area	28.28^{c}	6.91^{b}	3.15^{a}	0.19	9.37^{c}	6.95^{b}
No. of black spots	7.59^{b}	3.48^{a}	2.77	0.17	6.08^{b}	2.47
Structural colour area	13.59^{c}	4.82^{a}	4.30^{a}	2.59	4.78^{a}	2.51
No. of structural spots	3.18	0.80	3.40^{a}	1.22	1.71	0.33

Note: Shown are the *F*-statistics from analyses of variance, including effects of predation environment (high or low) and river (Aripo, Quare, Yarra). Significance levels are denoted by superscripts: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. Parallel evolution is revealed by a significant effect of predation and a non-significant predation × river interaction. In all cases, differences are in the same direction: low-predation males are larger and more colourful (see Fig. 1).

Table 3. Statistical analysis of male traits for wild-caught ($n = 20$) and laboratory-reared ($n = 10$) fish from paired high- and low-predation populations *within* each of three rivers

	Wild-caught			Laboratory-reared		
Trait	Aripo	Quare	Yarra	Aripo	Quare	Yarra
Body area	53.34^{c}_{L}	31.87^{c}_{L}	62.38^{c}_{L}	12.83^{b}_{L}	3.67	1.68
Tail area	14.21^{c}_{L}	15.45^{c}_{L}	15.90^{c}_{L}	2.24	6.15^{a}_{H}	1.71
Orange area	0.04	4.00^{a}_{L}	44.58^{c}_{L}	0.98	1.75	18.68^{c}_{L}
No. of orange spots	0.19	5.49^{a}_{L}	8.39^{b}_{L}	2.42	0.00	0.27
Black area	14.46^{c}_{L}	6.64^{a}_{L}	7.50^{b}_{L}	5.16^{a}_{L}	6.46^{a}_{H}	0.11
No. of black spots	4.99^{a}_{L}	6.61^{a}_{L}	0.14	6.92^{a}_{L}	1.11	0.03
Structural colour area	11.53^{b}_{L}	0.60	3.20	2.51	2.17	3.27
No. of structural spots	9.90^{b}_{L}	0.65	0.59	1.28	0.01	0.46

Note: Shown are *F*-statistics from one-way analysis of variance. Significance levels are denoted by superscripts: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. The population with the larger mean trait value is marked by the subscript 'L' (low-predation) or 'H' (high-predation).

level preference functions, although the strength of these functions varied among rivers (Table 5). Contradicting some previous work (see Discussion), we found no indication of female preferences for increased size or colour in any population. Instead, females from low-predation populations generally showed no discrimination with respect to male traits (i.e. flat preference functions). Females from high-predation populations, however, generally showed preferences for smaller trait values (i.e. less colour and smaller size). In general, preference functions significantly diverged between predation regimes for the area of orange, the number of black spots, and tail size. The likelihood of three (or more) tests out of ten being significant (at $P < 0.05$) by chance alone is only 0.0024 (Chapman *et al.*, 1999),

Table 4. Statistical analysis of linear preference functions for various male traits

Male trait	Trait	River	Predation	Predation × Trait	River × Trait × Predation
Display rate	15.72^{c}	2.73^{a}	0.68	0.01	1.12
Sneak rate	8.44^{c}	0.64	0.04	0.11	0.87
Orange area	2.70	0.85	5.78^{b}	5.22^{b}	0.65
No. of orange spots	0.91	1.19	3.09^{a}	0.55	0.51
Black area	5.14^{b}	0.21	3.04^{a}	1.64	0.15
No. of black spots	1.27	2.13	15.76^{c}	15.88^{c}	0.07
Structural area	0.72	0.77	0.07	0.19	0.05
No. of structural spots	3.90^{b}	0.57	1.75	1.21	0.73
Body area	2.49	2.73^{a}	4.28^{b}	4.35^{b}	4.11^{b}
Body length	1.93	2.70^{a}	2.21	2.32	5.08^{c}
Tail area	1.76	3.17^{b}	3.74^{b}	3.73^{b}	1.63
Tail length	1.37	0.16	7.06^{c}	6.19^{c}	2.83

Note: Shown are *F*-values from mixed-model (predation = fixed; river = random) analyses of covariance for all main effects and the interactions of interest. Significance levels are denoted by superscripts: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$. Parallel evolution is revealed by a significant predation × trait interaction.

Table 5. Directions of female preference functions for male traits in each population

	Aripo		Quare		Yarra	
	High	Low	High	Low	High	Low
Display rate	$-^{b}$	$-^{c}$	$-^{b}$	–	–	–
Sneak rate	–	–	$-^{a}$	–	–	$-^{a}$
Orange area	$-^{b}$	+*	–	+	–	+
No. of orange spots	$-^{a}$	+	–	+	+	+
Black area	–	+	$-^{a}$	+	+	+
No. of black spots	$-^{a}$	+	$-^{b}$	$+^{c}$***	–	+
Structural area	+	+	0	–	–	$-^{a}$
No. of structural spots	+	–	$-^{c}$	+	+	+
Body area	$-^{c}$	+**	+	+	–	+*
Body length	$-^{c}$	+***	+	+	–	+
Tail area	–	+	+	$+^{a}$	$-^{a}$	$+^{b}$**
Tail length	–	+	$-^{b}$	+**	$-^{c}$	+

Note: Shown is the sign of the regression coefficient (β), with the superscript indicating significance: $^{a}P < 0.5$; $^{b}P < 0.01$; $^{c}P < 0.001$. Asterisks indicate significant slope differences between high- and low-predation populations within a river (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

indicating that the results of the individual tests are directly informative for mate choice divergence. For these traits, high-predation females preferred males who were less colourful and smaller (Fig. 2). For the other traits, the direction and strength of female preference were typically variable among populations, and did not map closely on to predation environments. Consistent with the results of step-wise regressions (see above), this result suggests that female preferences may evolve in response to factors in addition to the crude high- versus low-predation contrast.

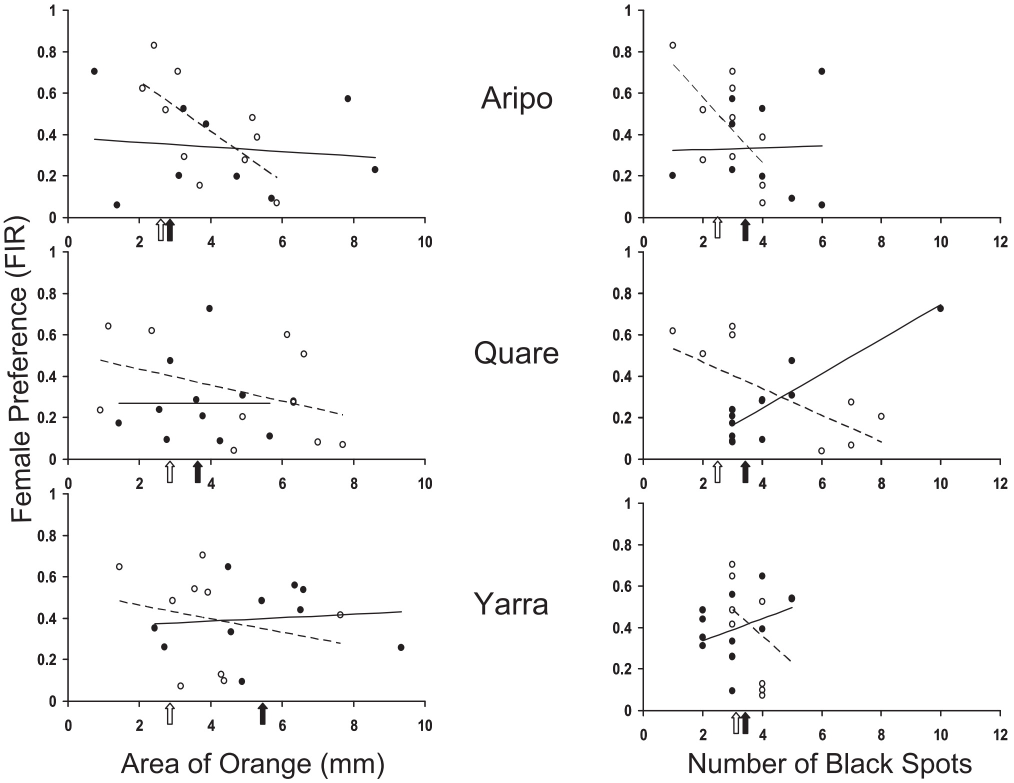
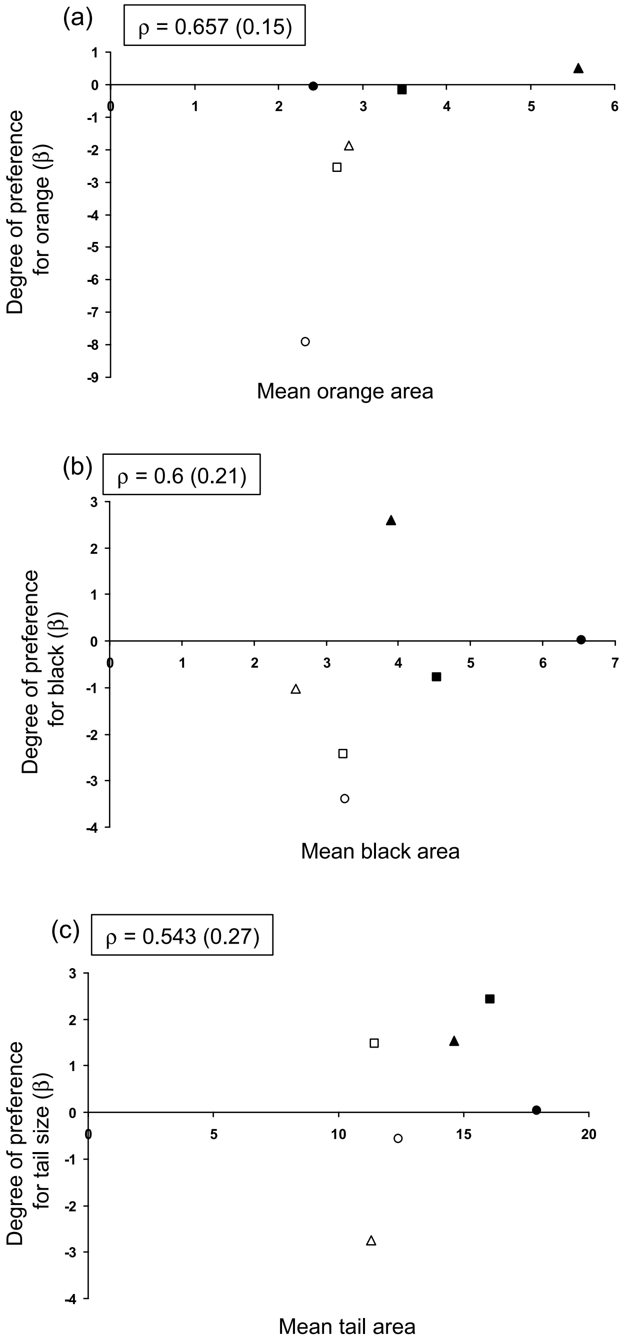


Fig. 2. Representative preference functions for high-predation (broken lines, open symbols) and low-predation (solid lines, solid symbols) populations in each river. Lines represent linear regressions of female preference on area of orange (left-hand panels) and black spot number (right-hand panels). Each point represents a female's response (FIR) to a male from her own population. Mean trait values for high- (open symbols) and low-predation (closed symbols) populations are indicated by arrows on the x-axis.

The above conclusions are confirmed by differences between predation regimes within each river (Table 5). In particular, although the *direction* of preference function divergence is often in parallel in the three rivers (less positive or more negative in high-predation populations), the *magnitude* of this divergence varies considerably for the same traits. Aripo females showed significant divergence in their preference for orange area ($F = 4.47$, $P = 0.05$), body length ($F = 8.76$, $P = 0.0009$), and body area ($F = 8.47$, $P = 0.01$). Quare females showed significant divergence in preferences for tail length ($F = 8.79$, $P = 0.008$) and the number of black spots ($F = 20.44$, $P = 0.0003$). Yarra females showed significant divergence in preferences for body area ($F = 4.5$, $P = 0.05$) and tail area ($F = 8.72$, $P = 0.01$).

Parallel co-evolution

The preceding results suggest that preference functions are *in general* evolving to match the direction of divergence of natural selection on male signalling traits between predation environments. Although low predation is selecting for more colour than high predation,



there are nonetheless differences in the average trait values among rivers (Fig. 1; Table 2), as well as in the strength of female preferences for these traits, even within a given predation type (Tables 4 and 5). We were therefore interested if differences in the degree of preference among populations were directly correlated with differences in male colour and size among

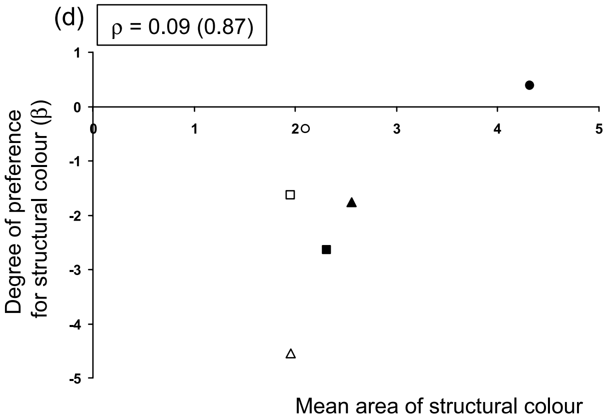


Fig. 3. Degree of female preference (slope of preference function) for (a) orange, (b) black, (c) tail area, and (d) structural colours as a function of the average trait value in the female's source population. Open and solid symbols refer to the high- and low-predation population in each river, respectively: Aripo = circles, Quare = squares, Yarra = triangles. The Spearman-rank correlation (ρ), with corresponding P-value (in parentheses), illustrates the strength of the overall correlation between trait and preference variation.

populations. We assessed co-evolution in this manner for two traits that showed parallel divergence in preference (orange and tail area), and two that did not (black and structural colour area). Figure 3 shows the overall pattern of correlation between the degree of preference for a given trait (slope of the regression coefficient, β) and the average male trait value from the female's population for the six populations. Indeed, female preferences tend to be higher in populations with higher average trait values, particularly for orange and black; however, this is mostly attributable to differences in preference among predation environments, rather than within them. Spearman rank correlation coefficients were all positive, but not significant.

DISCUSSION

Our first major conclusion is that divergent natural selection acting on Trinidadian guppies has led to the broadly parallel co-evolution of male secondary sexual traits and female preferences for those traits. Although previous work has shown that these traits can indeed co-evolve (Houde and Endler, 1990; Rodd *et al.*, 2002), our results show that this co-evolution occurs in parallel between predation regimes. With respect to male traits, guppies are generally more colourful in low-predation environments than in high-predation environments (Fig. 1; Tables 2 and 3). With respect to female preferences, guppies from high-predation environments discriminate against large and colourful males, whereas those from low-predation environments show no discrimination with respect to male traits (Fig. 2; Tables 4 and 5). The direction of divergence in female preferences therefore broadly matches the direction of divergence in male traits across three independent guppy lineages.

Our second major conclusion is that parallel co-evolution of male traits and female preferences is not very precise. With respect to male traits, although the direction of divergence was consistent, the extent of divergence between predation environments differed considerably among the three rivers (Tables 2 and 3). This was the case even for orange, which is traditionally thought to show reasonably consistent divergence between predation environments (Endler, 1980). These non-parallel aspects of divergence suggest that selection on male colour may depend on site-specific nuances of natural or sexual selection, rather than just the general contrast between 'low' and 'high' predation (see also Millar *et al.*, 2006). With respect to female traits, the slopes of preference functions varied among rivers within predation regimes (Fig. 3; Table 5), which again suggests the potential role of site-specific variation in selection.

Another possibility is that variations in female preferences among populations may impose different degrees or directions of sexual selection, resulting in divergence among populations independent of predation environment. Houde and Endler (1990) found strong evidence for the co-evolution of traits and preferences for orange colour; however, this pattern was mostly evident for populations that are naturally high in orange colour. Here, the associations between mean male trait values and the slopes of female preference functions were quite weak (Fig. 3), even for populations with high trait values. For example, although orange colour was not divergent between predation environments in the Aripo river, female preferences here showed their greatest divergence. Conversely, orange colour diverged dramatically in the Yarra river, but female preferences here showed their least divergence. On the one hand, this mismatch between male trait and female preference divergence may suggest that some aspects of female choice are conserved in both predation environments. For example, some male traits may reveal good genes benefits (*sensu* Zahavi, 1975; Houde and Torio, 1992; Grether, 2000; Evans *et al.*, 2004) or signalling biases (i.e. if more colourful males are simply more visible to females) that are reasonably consistent across environments. On the other hand, stronger divergence for male traits than for female preferences may suggest that divergent selection owing to predation acts more strongly on the former than the latter. Thus, although differences in predation intensity ('high' vs. 'low') clearly influence male traits and female preferences, other forces are likely also at work.

Natural and sexual selection: concordance or conflict?

The extent to which natural and sexual selection act in the same direction will profoundly influence the rate and magnitude of divergence between populations. In general, we found that the slopes of female preference functions for most relevant traits were negative in high-predation populations and flat in low-predation populations. These findings suggest that sexual selection complements natural selection in high-predation environments (enhancing divergence), and does not oppose natural selection in low-predation environments (having no additional effect on divergence). This interpretation leads to the need for further consideration of two points. One is why we failed to find evidence that females preferred greater colour, when this has been reported to be the case in many other studies (e.g. Kodric-Brown, 1985; Houde, 1987; Nicoletto, 1993; Grether, 2000). Another is why high-predation males still retain reasonable amounts of colour (Fig. 1) if both natural and sexual selection favour reduced colour.

The observed lack of female preferences for greater male colour could partly reflect our experimental design. First, our use of virgin females in a no-choice design may have increased individual variation among females in their responsiveness. The lack of discrimination among different males may be a consequence of individual variation in choosiness, or preferences among females (Godin and Dugatkin, 1995; Brooks, 2002). Furthermore, mate sampling and history, as well as mate-choice copying, were eliminated in our design, but could potentially affect individual female decisions, or result in a more unanimous decision as to what traits constitute an 'attractive' male (Rosenqvist and Houde, 1997; Brooks, 1996; Godin *et al.*, 2005). Second, with the exception of structural colours (blue, violet, and green), laboratory-reared males were more colourful and larger than wild-caught males from the same population (Figs. 1 and 2). If female preferences are based on absolute rather than relative differences among males (Lande, 1981), the experimental males may have surpassed some absolute colour threshold after which females no longer favour greater orange. Perhaps a different sort of design or analysis would have revealed more positive relationships between female preferences and male colour; although other experimental designs have yielded similar results (Houde and Endler, 1990; Endler and Houde, 1995; Brooks and Endler, 2001). Female preferences may actually be more variable than previously thought, even in low-predation environments. Increased colour in low-predation males is generally attributed to selection imposed by females; however, if female preferences are indeed weaker, or more variable here, the direction of causality may be reversed. Other environmental features could instead be selecting for increased colour in low-predation environments and females here are not driving the exaggeration of these traits, simply not opposing it.

We can see several possibilities why high-predation males retain reasonable amounts of colour despite selection against it. High-predation populations may receive considerable gene flow from upstream populations, where males are more colourful (e.g. Becher and Magurran, 2000; Crispo *et al.*, 2006). This not a universal explanation, however, because some colour is retained even far from low-predation sites (Endler, 1978, 1980; Millar *et al.*, 2006; present study). Greater colour may carry other benefits even in high-predation populations. For example, colour could signal parasite resistance (Grether, 2000; Van Oosterhout *et al.*, 2003), females may prefer different suites of traits under different lighting conditions (Gamble *et al.*, 2003), or females may prefer to mate with 'novel' males, or those showing rare colour patterns (Hughes *et al.*, 1999; Punzalan *et al.*, 2005). Similarly, predators may have search images that target more common males (Olendorf *et al.*, 2006). Given these multiple possibilities, the maintenance of colour polymorphism in guppies remains an open question, yet it appears that the combined effects of dispersal and temporal variations in both natural and sexual selection could explain the maintenance of colour, even when generally costly (Brooks, 2000, 2002).

Implications for reproductive isolation and ecological speciation

Reproductive isolation among closely related taxa can be greatly influenced by divergence in male traits and female preferences for those traits (Lande and Kirkpatrick, 1988; Coyne and Orr, 2004). If divergent natural selection causes the co-evolution of these traits and preferences, then it could play a substantial role in ecological speciation (Mayr, 1963; Schluter, 2000; Nosil *et al.*, 2002; McKinnon *et al.*, 2004; Boughman *et al.*, 2005). In particular, co-evolution of traits and preferences can mean that gene flow between ecological environments is impeded by both natural and sexual selection (Lande, 1982; Servedio, 2004); migrants have both low survival and low mating success. Our results, together with those of other work, shed some light on this possibility in guppies.

In guppies, gene flow between predation environments will mostly be the result of low-predation guppies moving downstream over waterfalls and into high-predation environments (Becher and Magurran, 2004; Crispo *et al.*, 2006). In this context, selection imposed by predators should generally act against the migrants (Endler, 1980), except perhaps in the case of strong negative frequency-dependent predation (Olendorf *et al.*, 2006). Indeed, recent experimental work confirms the idea that survival in high-predation environments will be much lower for low-predation immigrants than for high-predation immigrants (D. Weese and M. Kinnison, unpublished data). Our data suggest that these migrants, males at least, might also be disadvantaged in mating. In particular, we found that high-predation females prefer less colourful males, which would disfavour the more colourful males migrating from low-predation populations. Natural and sexual selection may therefore act together in reducing gene flow between predation environments, leading to ecological speciation.

Despite this logic and evidence, the process of ecological speciation appears weak in guppies (Magurran, 1998, 2005). First, female guppies show only weak preferences for males from similar selective environments over those from different selective environments (Endler and Houde, 1995). Second, there is no indication of post-copulatory incompatibilities among populations from different selective environments (Endler, 1995), although such evidence is accumulating among guppy populations from different drainages (Alexander and Breden, 2004; Russell and Magurran, 2006; Ludlow and Magurran, 2006). Third, neutral gene flow is not reduced between sites in different selective environments relative to between sites in similar selective environments (Crispo *et al.*, 2006). This paradox requires explanation. One possibility is that some females in high-predation environments may prefer colourful males (see above), in which case the survival disadvantage of migrant males may be offset by a mating advantage. Another possibility is that female choice may be circumvented through sneaky copulations by males in high-predation environments (Magurran, 1998, 2005). Post-copulatory mechanisms may therefore be important in influencing the actual relative mating success of migrant and resident males (Evans and Magurran, 2001; Pilastro *et al.*, 2002). More studies directly comparing mating success in the wild and in the laboratory are therefore needed to help understand this paradox.

Summary and prospects

Our results suggest that local adaptation to divergent predation regimes has led to the broadly parallel co-evolution of male traits and female preferences in Trinidadian guppies. The resulting female choice for locally adapted phenotypes, at least in high-predation populations, should enhance divergence in male secondary sexual traits (e.g. Schluter and Price, 1993; Boughman, 2001; Klappert and Reinhold, 2005). However, we also found evidence for deviations from closely parallel co-evolution, suggesting that the simple high- versus low-predation contrast may not tell the full story when it comes to the evolution of male colour and female preferences in Trinidadian guppies.

Almost all work on ecological speciation so far has been confirmatory (for reviews, see Schluter, 2000; Rundle and Nosil, 2005). One might have predicted that guppies would add to this pantheon given that they experience very strong divergent selection between predation regimes, and that male sexual traits and female preferences co-evolve in parallel between these environments. Yet, the process of ecological speciation appears very weak in guppies (Magurran, 2005; Crispo *et al.*, 2006). The greatest progress towards understanding ecological speciation may perhaps now come from studies that attempt to understand the conditions that both drive and limit the influence of natural selection on the evolution of reproductive isolation.

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