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This is *not* déjà vu all over again: male guppy colour in a new experimental introduction

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Abstract

We use an experimental introduction in nature to examine factors that influence parallel evolution. In 1996, 200 high-predation guppies (*Poecilia reticulata*) from the Yarra River were introduced into the Damier River, which previously lacked guppies. Eight years later, we quantified the colour of wild-caught guppies ('phenotypic' divergence) and lab-reared guppies ('genetic' divergence) from low- and high-predation environments in both rivers. Phenotypic and genetic divergence between predation environments *within* the Yarra was evident for black and for orange. Phenotypic divergence *within* the Damier was parallel to the Yarra for black but not for orange. Genetic divergence was absent between predation environments *within* the Damier, but was evident when comparing both Damier populations to their Yarra ancestors. The evolution of male colour thus depends on factors other than the simple contrast between 'high' and 'low' predation. We suggest that the parallel evolution of male signalling traits may sometimes first require the parallel evolution of female preferences.

Introduction

When different populations or species colonize similar environments, they often evolve similar adaptive solutions (Williams, 1972; Jones *et al.*, 1992; Reid *et al.*, 2000; Schluter, 2000; Langerhans & DeWitt, 2004; Melville *et al.*, 2006). This replicated adaptation is considered 'parallel' when it evolves from similar ancestors and 'convergent' when it evolves from different ancestors. The evidence for parallel/convergent evolution spans a wide range of organisms (plants, animals, bacteria), taxonomic scales (conspecific populations, closely related species, completely different lineages) and selective agents (e.g. temperature, latitude, predation, competition). This phenomenon therefore provides a useful substrate for examining the role of natural selection as a driving force in evolutionary diversification. In the present manuscript, we use the colour of male guppies (*Poecilia reticulata*) as a way of addressing three questions that inform the generality, power and nuances of parallel/convergent evolution.

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Is parallel/convergent evolution predictably driven by broad-brush contrasts in selection?

This question is often answered in the affirmative (e.g. Langerhans & DeWitt, 2004; Langerhans *et al.*, 2006; Melville *et al.*, 2006), with particularly strong evidence coming from Poecillid fishes, including guppies. In particular, Poecillids in low- vs. high-predation environments often show predictable adaptive divergence in colour (Endler, 1982), life history (Reznick & Bryga, 1996; Reznick *et al.*, 1996), behaviour (Magurran, 1990; Magurran *et al.*, 1992; Magurran, 2005) and morphology (Langerhans & DeWitt, 2004; Hendry *et al.*, 2006). This repeatable divergence is evident despite substantial variation in the *specific* predator species, implying that adaptive evolution is driven by the broad-brush difference in predation *intensity*. And yet, parallel/convergent evolution is rarely perfect; i.e. populations in similar

environments often deviate from each other in some aspects of their phenotype (Reznick & Bryga, 1996; Reznick *et al.*, 1996; Gilchrist *et al.*, 2004; Langerhans & DeWitt, 2004; Langerhans *et al.*, 2006; Melville *et al.*, 2006). One potential explanation for these deviations is variation among local populations in the nuances of selection within a particular 'environment'. We address this question by comparing male colour divergence between high- and low-predation environments in rivers with different predator species and different habitat features.

How quickly can selection drive adaptive divergence?

Human-mediated introductions have often been used to examine rates of evolution (reviews: Hendry & Kinnison, 1999; Kinnison & Hendry, 2001; Stockwell *et al.*, 2003). Such studies often find that introduced populations can undergo substantial contemporary evolution in the direction predicted from patterns seen in long-standing populations. Some of our favourite examples include wing size clines in *Drosophila* (Gilchrist *et al.*, 2004), reproductive investment in chinook salmon (Kinnison *et al.*, 2001), armour traits in threespine stickleback (Bell, 2001), and colour and life history in guppies (Endler, 1980; Reznick *et al.*, 1997). In fact, some of the fastest rates of evolution on record are those for male guppy colour in Endler's (1980) experiment (see Hendry & Kinnison, 1999). Interestingly, this classic experiment is one of only a few studies of how quickly secondary sexual traits evolve in natural populations (Svensson & Gosden, 2007). It therefore seems useful to determine whether Endler's (1980) results are anomalous or typical. We here address this question by comparing male colour divergence in a new experimental introduction to that seen by Endler (1980).

How is parallel/convergent evolution influenced by sexual selection?

Most studies of parallel evolution focus exclusively on the role of natural selection. For secondary sexual traits, however, evolution may often reflect a balance between sexual selection favouring conspicuousness and natural selection favouring crypsis (Endler, 1980; Andersson, 1994). In some cases, such as guppies (see below), the colonization of new environments may relax natural selection on secondary sexual traits, which then allows their exaggeration through the action of sexual selection. In these cases, evolution will depend on the nature of sexual selection in the ancestral population (and therefore in the colonists), as well as how sexual selection changes following the introduction. We address this question by considering why male guppy colour does not always increase following the introduction of high-predation fish into a low-predation environment (see below).

Trinidadian guppies

Wild guppies live in a variety of conditions. Downstream 'high-predation' populations are typically exposed to 'dangerous' predatory fishes that have a major impact on guppy demographics. In contrast, upstream 'low-predation' populations are typically exposed to only weak predatory fishes. The resulting difference in predation intensity is associated with a broad suite of adaptive differences between high- and low-predation guppy populations (Endler, 1995; Reznick *et al.*, 1996; Houde, 1997; Magurran, 2005). With respect to life history, low-predation females mature later and produce fewer but larger offspring (Reznick & Bryga, 1996; Reznick *et al.*, 1996; Reznick *et al.*, 1997). With respect to behaviour, low-predation fish spend more time foraging and less time shoaling, and also show less careful predator inspection (Magurran, 1990; Magurran *et al.*, 1992; Magurran & Seghers, 1994). With respect to secondary sexual traits, low-predation males are often more colourful (Haskins *et al.*, 1961; Endler, 1978, 1980; Winemiller *et al.*, 1990; Millar *et al.*, 2006). This last difference is thought to have arisen because females often prefer to mate with more colourful males (Kodric-Brown, 1989; Houde & Torio, 1992; Houde, 1997), whereas predators select against such males in high-predation environments (Haskins *et al.*, 1961; Endler, 1980; Millar *et al.*, 2006). The low-predation populations in each river are thought to have evolved separately from the high-predation populations in that same river, and the replicated divergence is therefore considered 'parallel'.

Thus unfolds the guppy story as classically envisioned, and yet the full picture is heavily nuanced by additional selective factors (Endler, 1983; Grether *et al.*, 2001; Reznick *et al.*, 2001; Magurran, 2005; Millar *et al.*, 2006). We here give a flavour for some of this complexity by reference to our trait of interest: male colour. Different suites of predators are found in different parts of Trinidad, such as the north vs. south slopes of the Northern Mountain Range, and these predators have different visual sensitivities. On the one hand, crustaceans (prawns, *Macrobrachium* sp.) are much more abundant on the north slope than on the south slope (Millar *et al.*, 2006; N. Millar, unpublished data), and these predators are insensitive to long wavelengths of light (orange/red). On the other hand, cichlid fishes (e.g. *Crenicichla alta)* are abundant on the south slope but are absent from the north slope (Endler, 1983; Reznick *et al.*, 1996), and these predators are insensitive to short wavelengths of light (ultraviolet). Consistent with the expectation that the visual sensitivity of predators will influence selection on colour, this trait is correlated with the presence or abundance of specific predator species (Endler, 1978; Millar *et al.*, 2006). In addition, habitat features, such as stream size and canopy openness, vary dramatically among guppy populations (Magurran, 2005), and some of these features appear to influence

male colour (Grether *et al.*, 1999; Millar *et al.*, 2006). This complexity suggests that male guppy colour is a useful system in which to examine the details of parallel/convergent evolution, as well as deviations there from.

Our study

We focused on the Yarra and Damier rivers, which are located next to each other on the north slope of the Northern Range mountains of Trinidad (Fig. 1). By the early 1990s, several years of surveys by D.N. Reznick (personal communication) confirmed that the Damier River completely lacked guppies, as is also the case for some other small rivers on the north slope (Magurran, 2005, p. 12). In the mid-1990s, D.N. Reznick twice attempted to introduce Yarloraw-predation guppies into the high-predation environment of the Damier. In each case, subsequent surveys showed that these fish had disappeared, as is predicted based on population dynamic models (Reznick *et al.*, 2004). Then, in 1996, D.N. Reznick (personal communication) haphazardly selected 200 guppies from a site in the lower reaches of the Yarra River (grid reference PS 804 940). At this site, guppies coexist with dangerous fish predators and exhibit the classic high-predation life history (Reznick *et al.*, 2004, 2006). These guppies were introduced into a low-predation site in the Damier River – above a barrier waterfall that prevents upstream colonization by dangerous fish predators. When the Damier River was surveyed 1 year after this last introduction, guppies were very abundant in the low-predation environment, and had also moved downstream over the waterfall to become abundant in the high-predation environment (D.N. Reznick, personal communication). These observations, in conjunction with the pristine nature of the Damier watershed (it is preserved as the water source for the town of Blanchisseuse), make us confident that this specific introduction was the source of guppies currently found in the Damier.

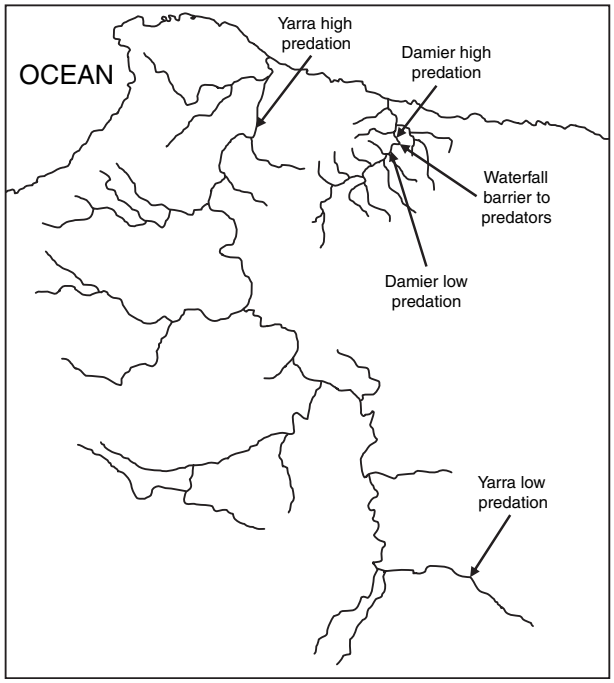

Fig. 1 Map of the Yarra and Damier rivers. Shown are the study populations, and the waterfall that separates low- and high-predation sites in the Damier.

The two environments in the Damier currently mirror the typical high- vs. low-predation contrast. In our many visits to this river, we have often seen dangerous predators (e.g. *Dormitator maculatus, Eleotris pisonis, Gobiomorus dormitor*) below the waterfall, but never above it. Moreover, mark–recapture experiments confirm that mortality rates are higher below the waterfall than above it, and that resident guppies in the two environments have diverged as expected for life history phenotypes (S. Gordon, A. Hendry and D. Reznick, unpublished data). The present study is based on guppies collected from these sites in 2004, 8 years after the original introduction. This time span represents 26 generations if we assume a typical generation length for high-predation guppies and 13 generations if we assume a typical generation length for low-predation guppies (Reznick *et al.*, 1997).

Materials and methods

Wild-caught males

In April of 2004, we collected adult males from four locations (Fig. 1): Yarra high predation (YH, the ancestral population), Yarra low predation (YL), Damier high predation (DH) and Damier low predation (DL, introduction site). Each male was anaesthetized with MS-222 (tricaine methane sulphonate), illuminated with full-spectrum fluorescent bulbs and photographed (Nikon Coolpix 995, Nikon Canada Inc., Mississauga, ON, USA) on a standard background. Two photographs were taken of each fish, one with the camera's flash and one without it (see Millar *et al.*, 2006 for details).

The quantification of colour pattern followed Millar *et al.* (2006). In brief, Scion Image (version Alpha 4.0.3.2; Scion Digital Imaging Software, Frederick, MD, USA) was used to measure (i) the area of the left side of the body and (ii) the area of each colour spot on the left side of the body. These area measurements excluded the tail and fins, because their fragility prevents reliable positioning for photographs. Each spot was visually assigned to one of seven colour categories: orange (includes red), black (includes fuzzy black), violet-blue (includes iridescent), yellow, bronze-green, green and blue. Violet-blue and blue were difficult to reliably differentiate on some of the photographs, and so were combined for analysis (henceforth called 'blue'). The photographs were analysed in random order by a single person (NK) who did not know

the population of origin for a given fish. Sample sizes were 100 males for YH, 101 for YL, 95 for DH and 98 for DL.

We here focus on three colour categories of known biological relevance (orange, black and blue), as well as the total amount of colour (all colours pooled). For each of these colour categories, we follow previous work (Endler, 1978, 1980; Millar *et al.*, 2006) in analysing several different metrics: (i) the total area of a given colour divided by the total body area of the fish ('relative area' of colour), (ii) the number of spots of a given colour ('number of spots') and (iii) the average area of individual spots of a given colour divided by the total body area of a fish ('relative size' of spots). Relative values from (i) and (iii) were arcsine square root transformed for statistical analysis.

Statistical analyses were separate for each metric in each of the four colour categories. Colour variation in these wild-caught males was first analysed by using two-way ANOVAS, in which river (Yarra or Damier) and predation regime (low or high) were fixed factors. Frequent interactions between these factors (*Supplementary Material*, Table S1) precluded meaningful interpretations of main effects. We therefore next analysed each of the four groups (YH, YL, DH, DL) as separate levels of a single factor in one-way ANOVAS, followed by *post hoc* Student–Neuman–Keuls tests. This particular *post hoc* procedure was used because Tukey tests resulted in too many type II errors: e.g. significant differences in the overall ANOVA but not in the *post hoc* comparisons.

Lab-reared males

In April of 2004, 15–20 pregnant females were captured from each of the four locations (Fig. 1) and brought back to our laboratory at McGill University, where they were isolated until they gave birth. When the resulting 'F1' offspring matured, they were mated within each population in a randomized design that excluded brother–sister pairings. The resulting 'F2' offspring were unlikely to manifest any plastic or maternal effects because of their original environments (Reznick, 1983). We therefore refer to divergence based on lab-reared males as 'genetic'. Each F2 litter was reared in its own aquarium until the sex of individuals could be determined. Two males from the middle of the size distribution in each litter were then isolated and placed into individual aquaria, with one male being fed a 'low food' diet and the other a 'high food' diet (following Reznick, 1983). This diet manipulation was performed because resource levels can influence the evolution and plastic expression of male colour (e.g. Grether *et al.*, 2001).

Each F2 male was checked daily to see if it was mature, as indicated by a fully formed gonopodium (a curved hook at the distal tip of the third and fourth anal fin rays, Winemiller *et al.*, 1990). Within a week after maturing, each F2 male was anaesthetized, photographed and analysed for colour in the same way as the wild-caught males (see above). Total sample sizes were 22 males for YH, 24 for YL, 22 for DH and 26 for DL, which included eight complete high-low food sibling pairs for YH, 10 for YL, 11 for DH and eight for DL. Colour increases with male age, although this ontogenetic variation does not seem to obscure genetic differences (Miller & Brooks, 2005). As in this previous work, we found age-related changes in male colour by repeatedly photographing the same individual over several weeks that did not substantially alter the observed patterns in comparisons among our four populations (N. Karim and A. Hendry, unpublished data). It remains possible, however, that some of the observed patterns might be different if we had measured the males at a different age.

Statistical analyses were separate for each metric in each of the four colour categories (similar to the above analyses for wild-caught males). We first used repeated measures ANOVAS to test for the effects of food level ('subjects' were sibling pairs on low vs. high food), river (fixed effect) and predation regime (fixed effect), along with all possible interactions. Because interactions between river and predation were common (*Supplementary Material*, Tables S2–S4), we reran the analyses using the four populations as separate levels of a single factor in ANOVAS that continued to include food level as a repeated measure. These analyses were followed by Student–Neuman–Keuls *post hoc* comparisons among the four populations.

Results

For the wild-caught males, and then for the lab-reared males, we first describe significant aspects of divergence between low- and high-predation regimes within each river, and then between the ancestral Yarra population (YH) and the two derived Damier populations (DH and DL). We then synthesize these results so as to more directly inform our specific research questions. In the following descriptions, 'marginal' is used in cases when *post hoc* comparisons could not completely eliminate type II errors.

Wild-caught males

Divergence within each river.

In the Yarra, YL males had twice as much relative orange area as did YH males, but the two populations did not differ in relative areas of the other colours (Fig. 2). Other significant differences in the Yarra were that YL males had fewer spots (except for orange, Fig. 3) and larger spots (only marginal for blue, Fig. 4). In the Damier, DL males had about 20% more relative blue area than did DH males, but the two populations did not differ in relative areas of the other colours (Fig. 2). Other significant differences in the Damier were that low-predation males had fewer black and orange spots (Fig. 3), and marginally larger black and blue spots (Fig. 4).

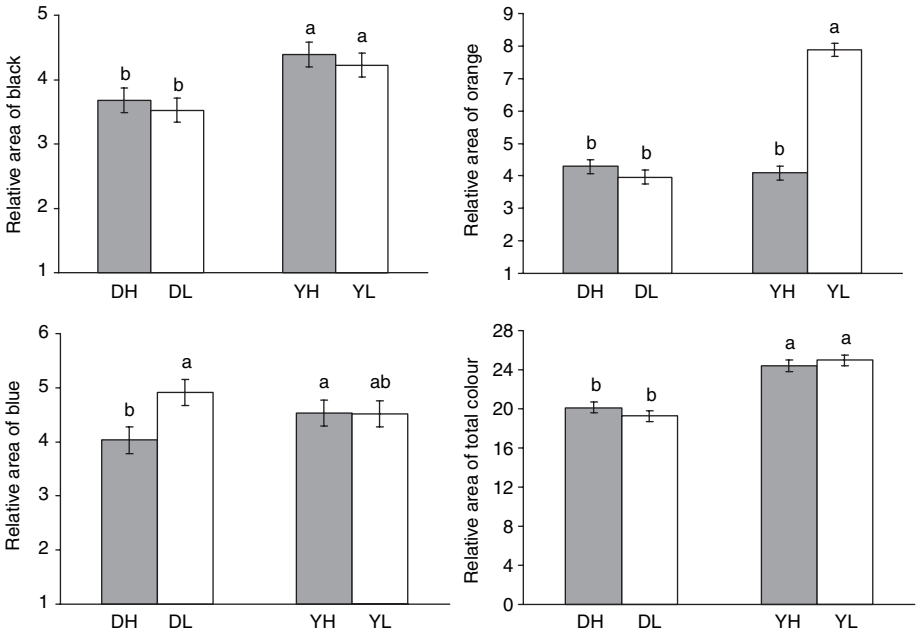

Fig. 2 Relative areas of selected colours for *wild-caught males* from each population: Damier high predation (DH), Damier low predation (DL), Yarra high predation (YH), Yarra low predation (YL). Shown are averages and standard errors from ANOVAS conducted on arcsine square root transformed values. Results of single factor ANOVAS are: black, $F_{3,390} = 5.029$, $P = 0.002$; orange, $F_{3,390} = 89.057$, $P < 0.001$; blue, $F_{3,390} = 3.321$, $P = 0.020$ and total colour, $F_{3,390} = 26.943$, $P < 0.001$. Letters (a,b,c) above the bars indicate Homogenous subsets as revealed by Student–Newman–Keuls tests. For clarity, 'a' is given above all bars when no significant differences are evident.

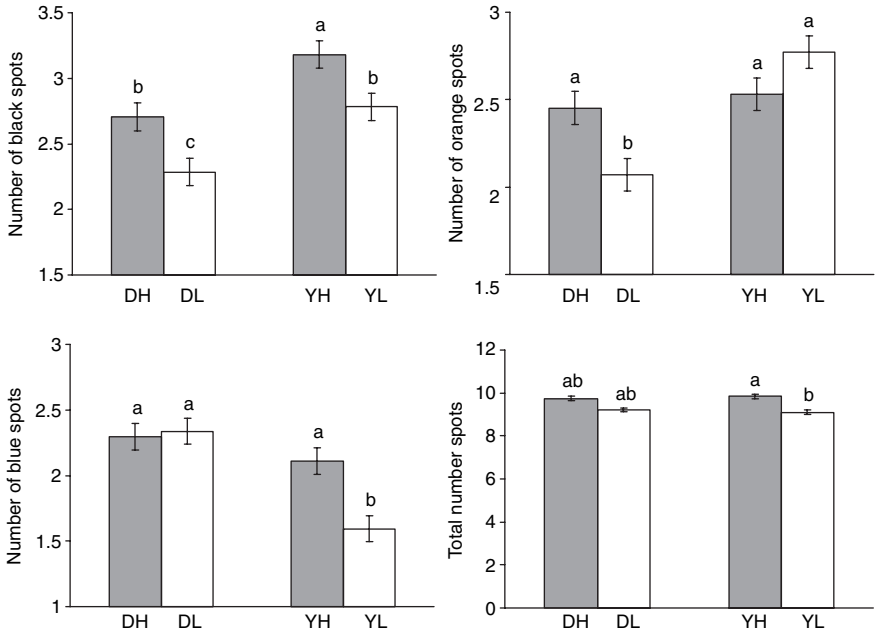

Fig. 3 Numbers of spots of selected colours for *wild-caught males*. All conventions are as described in the caption for Fig. 2, except that the data here are not arcsine square root transformed. Results of single factor ANOVAS are: black, $F_{3,390} = 13.475$, $P < 0.001$; orange, $F_{3,390} = 8.945$, $P < 0.001$; blue, $F_{3,390} = 12.006$, $P < 0.001$ and total colour, $F_{3,390} = 3.393$, $P = 0.018$.

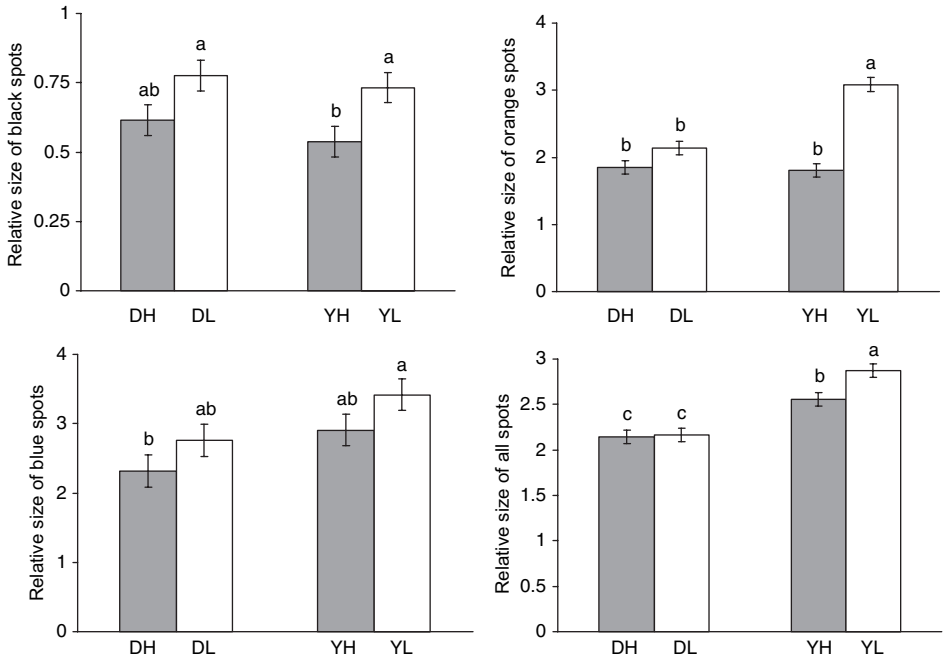

Fig. 4 Relative spot sizes of selected colours for *wild-caught males*. All conventions are as described in the caption for Fig. 2. Results of single factor ANOVAS are: black, $F_{3,390} = 4.043$, $P = 0.008$; orange, $F_{3,390} = 34.597$, $P < 0.001$; blue, $F_{3,390} = 23.852$, $P = 0.007$ and total colour, $F_{3,390} = 23.852$, $P < 0.001$.

Divergence between the ancestral and derived populations.

For relative areas of colour (Fig. 2), significant differences were: (i) both DH and DL males had less black and less total colour than did YH males and (ii) DH males had less blue than did both DL and YH males. For spot numbers (Fig. 3), significant differences were: (i) both DH and DL males had fewer black spots than did YH males and (ii) DL males had fewer orange spots than did both DH and YH males. For relative spot sizes (Fig. 4), significant differences were: (i) DH and DL males had smaller spots for all colours combined than did YH males, (ii) DH males had marginally smaller blue spots than did both DL and YH males and (iii) Both DL and DH males had smaller spots than did males.

Lab-reared males

Divergence within each river.

In the Yarra, YL males had more than twice as much relative orange area as did YH males, but the two populations did not differ in relative areas of the other colours (Fig. 5). This result closely matches that for the wild-caught males. For spot numbers, YL males had more orange spots than did YH males, but the two populations were otherwise generally similar (Fig. 6). The statistical significance of these results did not match those for wild-caught males (Fig. 3), but some trends were in the same direction, including more orange spots and fewer and larger black spots for YL males. For relative spot sizes, YL males had larger orange spots than did YH males, but the two populations were otherwise similar (Fig. 7). These patterns generally match those for wild-caught males, except that the difference for black in wild-caught males was not significant here (Fig. 4). In the Damier, no significant differences were evident based on any metric for any colour, although the trend for DH males to have less relative blue area than DL males in the wild-caught fish was in the same direction here.

Divergence between the ancestral and derived populations.

For relative areas of colour, the only significant difference was that DH and DL males had marginally less total colour than did YH males (Fig. 5). The difference in black area observed for wild-caught fish (lower in the Damier) was in the same direction here, but was no longer significant (Fig. 5). No significant differences were evident for spot number (Fig. 6) or relative spot size (Fig. 7), but the trends for black in wild-caught males (fewer and larger in the Damier) were in the same direction here.

Synthesis of the key results

The greatest difference between predation regimes in the Yarra (greater orange in the low-predation site) was

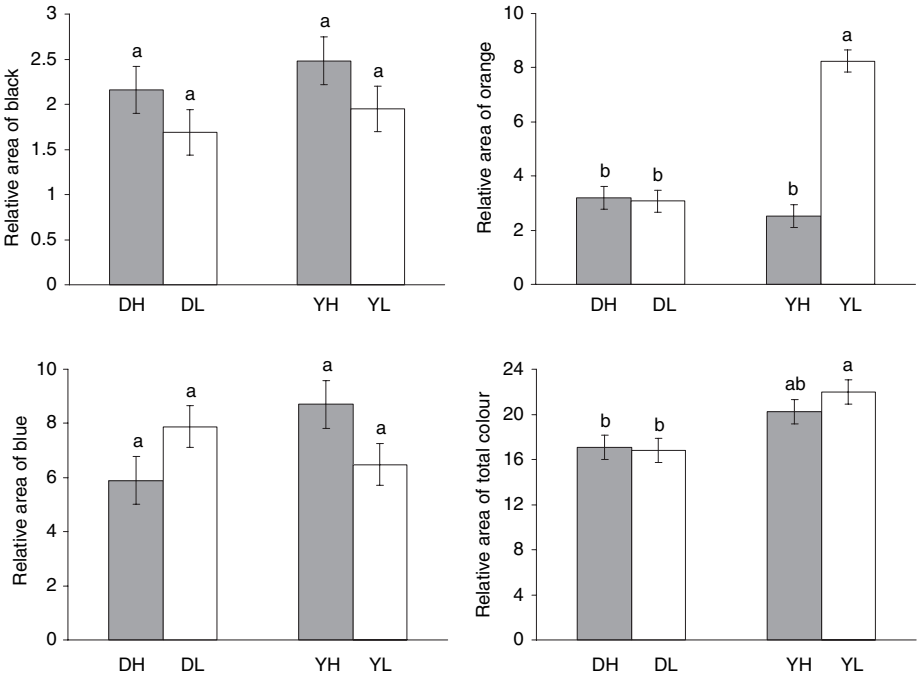

Fig. 5 Relative areas of selected colours for *lab-reared males*. All conventions are as described in the caption for Fig. 2. Results of single factor ANOVAS are: black, $F_{3,33} = 0.622$, $P = 0.606$; orange, $F_{3,33} = 34.918$, $P < 0.001$; blue, $F_{3,33} = 0.975$, $P = 0.416$ and total colour, $F_{3,33} = 0.690$, $P = 0.564$.

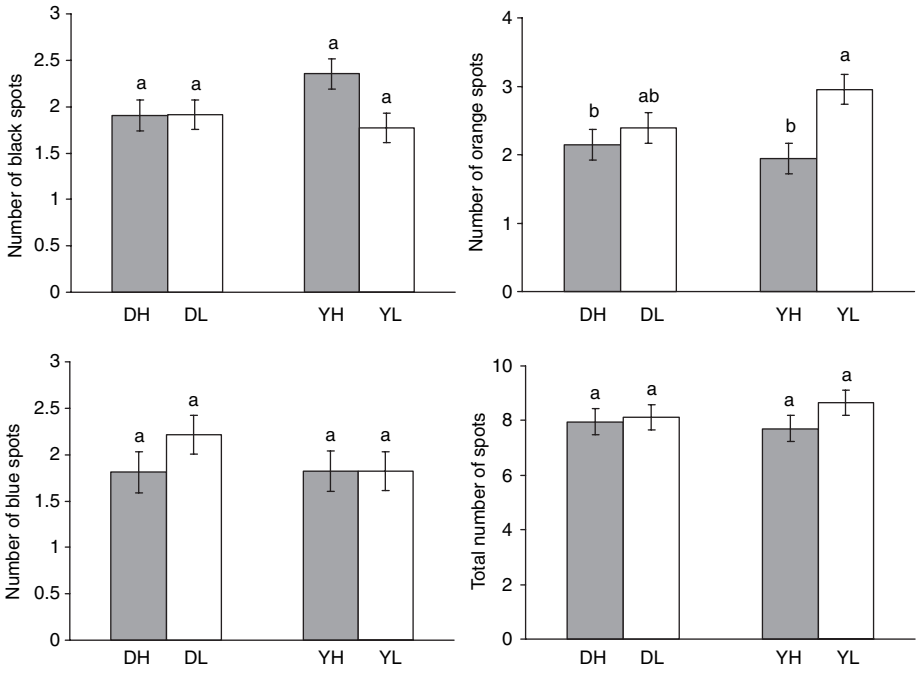

Fig. 6 Numbers of spots of selected colours for *lab-reared males*. All labelling conventions are as described in the caption for Fig. 2, except that the data are not arcsine square root transformed. Results of single factor ANOVAS are: black, $F_{3,31} = 0.785$, $P = 0.512$; orange, $F_{3,33} = 5.154$, $P = 0.005$; blue, $F_{3,30} = 0.573$, $P = 0.637$ and total colour, $F_{3,33} = 0.792$, $P = 0.507$.

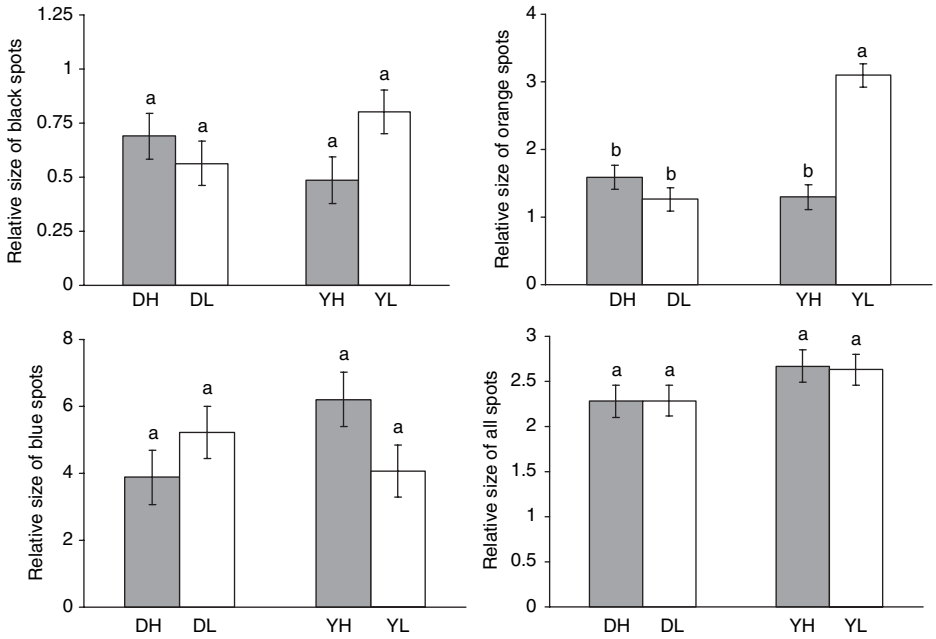

Fig. 7 Relative spot sizes of selected colours for *lab-reared males*. All labelling conventions are as described in the caption for Fig. 2. Results of single factor ANOVAS are: black, $F_{3,31} = 0.931$, $P = 0.437$; orange, $F_{3,29} = 22.816$, $P < 0.001$; blue, $F_{3,32} = 1.159$, $P = 0.340$ and total colour, $F_{3,33} = 1.805$, $P = 0.153$.

consistent for both wild-caught and lab-reared males, indicating its strong genetic basis. In contrast, no divergence in orange was evident within the Damier, nor between the Damier populations and their Yarra high-predation ancestors. Orange therefore does not appear to have changed appreciably following introduction to the Damier. Another divergent colour in the Yarra was black, with fewer but larger spots in the low-predation site. The magnitude of this difference was similar in wild-caught and lab-reared males, but was not significant in the latter, presumably because of the smaller sample sizes. In the Damier, the number and size of black spots diverged in parallel to the Yarra for wild-caught males but this difference was not evident in lab-reared males. Divergence for black in wild-caught Damier males may therefore have a plastic basis. Divergence between the Damier populations and their YH ancestors was greatest for black, with fewer but larger spots in the former. This difference was in the same direction for wild-caught and lab-reared males, but was smaller and nonsignificant in the latter. To summarize the above, the only colour to change substantially following introduction to the Damier was black. The changes in this colour appear to partly reflect plasticity-driven divergence between predation regimes (i.e. evident in wild-caught males but not in lab-reared males), as well as genetically based divergence between rivers (i.e. largely evident in both wild-caught and lab-reared males).

Discussion

Is parallel evolution driven by broad-brush contrasts in selection?

This question can be informed by considering three possibilities. First, if colour divergence is driven by broad-brush contrasts in predation *intensity*, then adaptive divergence between low- and high-predation environments should be similar in all rivers. Secondly, if colour divergence is influenced by *specific* predator species, then divergence between low- and high-predation environments may differ between the north and south slopes – because these have very different suites of predators (Endler, 1983; Reznick *et al.*, 1996). Thirdly, if colour evolution is sensitive to environmental factors other than predation, divergence between low- and high-predation environments may differ between rivers on the same slope, such as the small Damier River and large Yarra River (Fig. 1).

Previous work sets the stage for evaluating these possibilities. In general, Poeciliid fishes are less colourful in the presence of 'dangerous' predatory fishes than in their absence (Endler, 1978, 1980, 1982; Winemiller *et al.*, 1990; Millar *et al.*, 2006). And yet, male colour also varies considerably among populations within each of these two broad predation categories (Endler, 1978; Millar *et al.*, 2006). Selective factors potentially influen-

cing these nonparallel aspects of divergence are several, and we now consider them in turn. First, specific predator species do not appear particularly important for relative orange area, because this aspect of male colour is greater for low- than for high-predation populations on both slopes (Endler, 1978; Winemiller *et al.*, 1990; Millar *et al.*, 2006). In contrast, specific predator species may indeed be important for relative blue area, which is greater for low- than for high-predation populations on the south slope (Endler, 1978), whereas the opposite is true on the north slope (Millar *et al.*, 2006; present study). Secondly, predator densities may be important because males have more orange and less blue at sites with more prawns (Millar *et al.*, 2006). Thirdly, habitat features may be important because, for example, guppies at sites with more open canopies have less black (Millar *et al.*, 2006).

Our results allow further insight. Most striking among these was that orange did not diverge between predation regimes in the Damier, a result that contrasts with the Yarra (Figs 2 and 5) and with other rivers (Endler, 1978, 1980, 1982; Winemiller *et al.*, 1990; Millar *et al.*, 2006). In contrast, the number and size of black spots on wild-caught males did diverge in parallel in the Damier and the Yarra, although these differences were lost in lab-reared males. Phenotypic divergence between predation regimes for black therefore seems to have a plastic basis. We can see several possible reasons for the apparent lack of genetic divergence in male colour between predation environments in the Damier. First, the *intensity* of predation in low- or high-predation sites may differ between the rivers because of currently unknown differences in predator densities. Secondly, any evolution of increased orange in the Damier low-predation environment might first require the evolution of increased female preference for orange (see below). Thirdly, the two predation environments in the Damier are reasonably similar in physical habitat features, such as width, depth, flow and canopy openness (S. Gordon, unpublished data). To the extent that these features influence colour divergence between predation regimes in other rivers (Grether *et al.*, 1999; Millar *et al.*, 2006), divergent selection may be weaker in the Damier. Fourthly, gene flow might constrain divergence of Damier high-predation males from low-predation males immediately upstream. Gene flow will not, however, explain why the Damier low-predation males have not evolved as expected for a low-predation population – because populations above waterfalls will only rarely receive migrants from below (Crispo *et al.*, 2006). Fifthly, additive genetic variation may be low for male colour in some guppy populations (Hall *et al.*, 2004).

The overall importance of the broad-brush contrast in predation environment vs. river-specific nuances of selection can be formally assessed on the north slope by comparing effect sizes (partial η^2) for river, predation and their interaction (Langerhans & DeWitt, 2004; *Supplementary Material*, Tables S2–S4). The main effect of river informs the importance of river-specific habitat features that are shared across predation regimes. The main effect of predation informs the importance of broad-brush contrasts in predation that are shared across rivers. The interaction between river and predation informs the importance of contrasts in predation environments that differ between rivers. Each of these components of diversification was sometimes more important than the others, although predation was rarely the most important (*Supplementary Material*, Tables S2–S4). These results illustrate that evolution, even in the classic context of male guppy colour, is heavily contingent on local nuances. Many of these nuances likely relate to the details of local selection, whereas others may reflect founder effects, genetic drift, or gene flow. In truth, evolution in most systems will almost certainly be the result of both general and specific aspects of selection, as well as other evolutionary forces (Gilchrist *et al.*, 2004; Langerhans & DeWitt, 2004; Langerhans *et al.*, 2006).

How quickly can selection drive adaptive divergence?

We here calculate rates of change (standard deviations per generation, 'Haldanes') for male colour when guppies are introduced to new environments. We separately calculated rates of 'phenotypic' divergence (based on wild-caught males) and 'genetic' divergence (based on lab-reared males) for the three relevant comparisons: DL vs. DH, DH vs. YH and DL vs. YH. Assuming 26 generations have passed, rates of phenotypic divergence were 0.001–0.031 (median = 0.010) and rates of genetic divergence were 0.0002–0.026 (median = 0.012) (*Supplementary Material*, Tables S5 & S6). For black, the only colour that showed substantial evolution in the Damier, rates of phenotypic divergence were 0.001–0.031 (median = 0.018) and rates of genetic divergence were 0.0002–0.026 (median = 0.014). In comparison with other guppy introductions (Endler, 1980; Magurran *et al.*, 1995; Reznick *et al.*, 1997; see Hendry & Kinnison, 1999), these rates are similar to those for behaviour (0.002–0.032) and life history (0.014–0.149), but are much lower (even if we instead assume only 13 generations) than those for male colour (0.267–0.742). Substantial colour divergence is clearly possible over the time frame we examined, and yet divergence between the low- and high-predation populations in the Damier was minor. These comparisons suggest something special about Endler's (1980) experiment, or about ours. One difference was the much shorter time frame for divergence in Endler's (1980) experiment – because evolutionary rates are known to decrease with increasing time interval (Hendry & Kinnison, 1999; Kinnison & Hendry, 2001). We feel that a more important difference, however, may relate to differences in sexual selection among introduced populations, a topic to which we now turn.

How is parallel evolution influenced by sexual selection?

The evolution of greater male colour in low-predation environments is presumably the result of sexual selection imposed by females (Houde & Endler, 1990; Endler & Houde, 1995) in the absence of strong opposing natural selection (Haskins *et al.*, 1961; Endler, 1980). This expectation becomes particularly interesting in the context of high-predation fish colonizing low-predation sites, which is both the expected direction of natural colonization (Crispo *et al.*, 2006) and the direction imposed in the Damier introduction. In such cases, the evolution of male colour in the low-predation population will initially depend on female preferences in the introduced fish. So what are these preferences? Although it is widely assumed that females generally prefer greater male colour, substantial geographical variation in preference is evident (Houde & Endler, 1990; Endler & Houde, 1995; Brooks & Endler, 2001). Indeed, one might expect weaker preferences in high-predation populations – because exercising choice in the presence of predators may be costly (Houde & Endler, 1990). Indeed, experiments in our laboratory have found that females from the Yarra high-predation site do not generally favour males with greater colour (Schwartz & Hendry, 2007).

Unless female preference has evolved following colonization of the Damier, we therefore would *not* expect male colour to increase in this population. Weak or absent preference evolution does seem possible given that artificial selection on female choice does not always produce an evolutionary response (Hall *et al.*, 2004), and that mate choice experiments in our laboratory have revealed similar preference functions in the ancestral Yarra and derived Damier populations (L. Easty, A. Schwartz and A. Hendry, unpublished data). The general lack of male colour divergence in the Damier is therefore consistent with what is known about female preference evolution. With greater time, however, female preferences may evolve in the Damier low-predation population so as to favour greater male colour, and we might then expect to see an increase in male colour. For the same reasons, it would useful to know the pattern of female preferences in the population that Endler (1980) introduced. These considerations show how the parallel evolution of secondary sexual traits can depend not only on changes in natural selection, but also on ancestral patterns of sexual selection, and on their rate of evolution in a new environment. We are not aware of any study that has yet examined the contemporary evolution of female preferences in the wild.

Conclusions

We examined the evolution of male colour in an introduction of high-predation guppies from one drainage (Yarra) into another (Damier). This introduction afforded an opportunity to examine whether adaptive divergence between predation environments would be parallel in the new and ancestral drainages. It was not. Indeed, the only evidence of parallel divergence (the number and size of black spots) was detectable for wild-caught males but not lab-reared males. In contrast, divergence in male colour was evident for both wild-caught and lab-reared males when comparing populations in the Damier to their ancestral population in the Yarra. Our results thus indicate that male guppy colour sometimes responds most strongly to selective pressures other than the simple presence or absence of dangerous predatory fishes. Some of these pressures may relate to variation in physical habitat factors, such as canopy openness. In addition, we suggest that the evolution of increased colour in low-predation populations may sometimes first require the evolution of female preferences. The legacy of sexual selection in ancestral populations may sometimes slow the pace of evolution for male secondary sexual characters in new populations.

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Supplementary Material

The following supplementary material is available for this article:

Table S1 Two-factor ANOVA results for *wild-caught males*, showing effects of river, predation level, and their interaction on various colour elements.

Table S2 Repeated measures ANOVA results for *relative areas of colour in lab-reared males*, showing effects of food level, river, predation intensity (high or low), and their interactions.

Table S3 Repeated measures ANOVA results for *numbers of spots in lab-reared males*, showing effects of food level, river, predation intensity (high or low) and their interactions.

Table S4 Repeated measures ANOVA results for *relative spot sizes in lab-reared males*, showing effects of food level, river, predation intensity (high or low) and their interactions.

Table S5 Rates of phenotypic divergence in Haldanes (standard deviations per generation) based on *wild-caught males*.

Table S6 Rates of 'genetic' divergence in Haldanes (standard deviations per generation) based on *lab-reared males*.

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