Background matching ability and the maintenance of a colour polymorphism in the red devil cichlid

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Keywords:
background matching; camouflage; colour polymorphism; evolution; Midas cichlid; predation.

Abstract

The evolution and maintenance of colour polymorphisms remains a topic of considerable research interest. One key mechanism thought to contribute to the coexistence of different colour morphs is a bias in how conspicuous they are to visual predators. Although individuals of many species camouflage themselves against their background to avoid predation, differently coloured individuals within a species may vary in their capacity to do so. However, to date, very few studies have explicitly investigated the ability of different colour morphs to plastically adjust their colouration to match their background. The red devil (*Amphilophus labiatus*) is a Neotropical cichlid fish with a stable colour polymorphism, with the gold morph being genetically dominant and having a myriad of documented advantages over the dark morph. However, gold individuals are much rarer, which may be related to their heightened conspicuousness to would-be predators. Here, we tested the ability of differently coloured individuals to phenotypically adjust the shade of their body colour and patterns to match their background. In particular, we filmed dark, gold and mottled (a transitioning phase from dark to gold) individuals under an identical set-up on light vs. dark-coloured substrates. We found that, in contrast to individuals of the dark morph, gold and mottled individuals were less capable of matching their body colouration to their background. As a result, gold individuals appeared to be more conspicuous. These results suggest that a difference in background matching ability could play an important role in the maintenance of colour polymorphisms.

Introduction

Colour polymorphisms are a striking example of biological variation and are observed across a wide range of taxa. The adaptive significance of colour polymorphisms, where two or more genetically determined colour morphs exist in a population (Huxley, 1955), remains a topic of contention. Indeed, the adaptive significance of colour polymorphs and the mechanisms facilitating their maintenance still continue to attract considerable research interest (Coyne & Orr, 2004; Gray & McKinnon, 2007; Roulin & Bize, 2007; Hancox et al., 2013). This attention has focussed mostly on the ecological significance of colour polymorphisms, as well as their potential role in sympatric speciation (Sinervo & Svensson, 2002; Gray & McKinnon, 2007; Forsman et al., 2008; McKinnon & Pierotti, 2010). Yet, in most species, the exact mechanisms maintaining colour polymorphs remain unknown.

Body colour often correlates with behavioural and physiological traits, such as reproductive strategy, aggression, immune function and stress response (Barlow, 1983; Sinervo & Svensson, 2002; Pryke & Griffith, 2006; Pryke et al., 2007; Dijkstra et al., 2008; McKinnon & Pierotti, 2010). Therefore, the processes maintaining morph frequencies are likely to involve complex interactions and correlational selection. One potential difference
occurring between colour morphs is their relative conspicuousness to predators. Predation pressure is a strong selective force and previous studies have demonstrated how it can contribute to the maintenance of morph frequencies (Losey et al., 1997). For example, in pea aphids (Acyrthosiphon pismum), predation by ladybird beetles (Coccinella septempunctata) interacts with parasitism from wasps (Aphidius ervi) to balance a red–green colour polymorphism (Losey et al., 1997). In other taxa, such as the guppy (Poecilia reticulata), predation impacts on the variation and distribution of colour morphs, with individuals being less conspicuous in areas where predation pressure is higher (Endler, 1980; Young et al., 2011).

In response to predators, many species have the capacity to plastically alter their body colour to match their background (Stuart-Fox & Moussalli, 2009; Endler, 1978). Habitat-specific background matching has been observed in a range of taxa, including invertebrates, fish, reptiles and birds (Reed & Janzen, 1999; Stuart-Fox et al., 2004; Whiteley et al., 2009). For example, in the freshwater sculpin (Cottus aleuticus), body colour is significantly correlated to local substrate colour, and sculpins can alter their body colour to match new backgrounds in a matter of months (Whiteley et al., 2009). In contrast, rapid background matching, a process most likely under neural control, is well documented in cephalopods and has also been observed in fish and reptiles (Hanlon & Messenger, 1988; Stuart-Fox et al., 2008; Hanlon et al., 2009; Clarke & Schluter, 2011). Background matching ability has important ecological and evolutionary implications, with slow or less precise individuals potentially more likely to be noticed by predators (Endler, 1980). In the context of colour morphs, differences in the ability to background match – and hence avoid detection by would-be predators – has the potential to play an important role in maintaining polymorph frequencies. Nevertheless, morph-specific responses to predation, for example via camouflage, have been largely unexplored.

The Midas cichlid species complex (Amphilophus spp.) is a group of closely related Neotropical fish, several of which are polymorphic in regard to body colour (Elmer et al., 2010). In these polymorphic species, the majority of individuals are ‘dark’, whereas approximately 10% of individuals are ‘gold’ in body colour (yellow through red) (Meek, 1907; Barlow, 1973, 1976). All individuals begin life with the dark body colouration. However, genetically ‘gold’ individuals generally start to change colour, from around three to 12 months of age, that is, with many ‘gold’ fish starting the transition prior to reaching sexual maturity. This occurs when fish begin to lose the melanophores in their skin, which then exposes the underlying gold colouration – a process that can take several days to months to complete (Barlow, 1983; Dickman et al., 1988). Gold colouration has a dominant genetic inheritance and some intraspecific advantages of being gold have been demonstrated, such as, an increased growth rate of gold-coloured individuals when raised with dark individuals, less aggression being directed to gold-coloured individuals when they comprise < 50% of the population, as well as gold individuals dominating dark ones of equal size in dyadic encounters (Barlow, 1973, 1983; Lehtonen, 2014). However, despite the advantages, there is still no clear explanation as to why gold individuals are so clearly less common than dark individuals in native Amphilophus populations (Barlow, 1976). Interestingly, in an introduced population in Australia with relaxed natural predation pressure, gold individuals are more common than their dark conspecifics, making up approximately 65–70% of the population (Sowersby, Lehtonen and Wong; unpublished data).

It has earlier been noted that individuals of the dark morph have an ability to alter the shade of their body colour and their patterning, a process that is limited in genetically gold individuals, even as juveniles (Barlow, 1976; Dickman et al., 1990). Nevertheless, although a handful of studies have considered a difference in predation level as a factor in maintaining the Midas cichlid colour polymorphism (Annett, 1989; Dickman et al., 1990; Maan et al., 2008; Maan & Sefc, 2013; Kusch & Meyer, 2014; Torres-Dowdall et al., 2014), potential differences between the colour morphs in their ability to alter their colouration to better match their background have so far been largely overlooked. In this study, we used the colour polymorphic red devil (Amphilophus labiatus, Günther, 1864) to test the background matching ability of individuals of the two colour morphs against contrasting natural backgrounds. We hypothesized that dark individuals would be better able to alter their body colour/shade to match their surrounding background than gold individuals. Such a difference in ability to adjust their colour/shade would allow individuals of the dark morph to more closely match their background, leaving individuals of the gold morph more conspicuous and potentially more susceptible to would-be predators – thus helping to explain their low frequency in nature.

**Materials and methods**

**Study species**

*Amphilophus labiatus* is a Neotropical fish native to the great lakes of Nicaragua (Lake Nicaragua and Lake Managua). The species has been introduced into Australia within the last 40 years, where it has established a breeding population in the man-made Hazelwood Pondage in south eastern Australia (NIWA, 2008). The fish used in this experiment (*n* = 70) were collected from Hazelwood and transported to Monash University, where they were maintained in four large, bare-bottomed flume tanks (~3000 L) at a temperature of 24 °C on a 12:12 day/night cycle and fed daily on commercial...
Capturing background matching on natural substrates

To address the question of whether colour morphs differ in their ability to match their background, we set up glass tanks (150L), with either dark or light substrate. Dark substrate consisted of fine gravel, pebbles and small cobbles whereas light substrate contained sand and pebbles. Within each tank, a smaller area was created using clear Perspex sheeting (20 cm × 10 cm × 30 cm) in order to constrain fish close to the front of the tank. Fish were placed individually into the smaller area and allowed five minutes to acclimate. Using a digital camera (Nikon D5200 Digital SLR), fish were video recorded in high definition for a period of 30 s, with manual white balance and identical camera settings for each fish. Digital images are commonly used to quantify colouration in a range of taxa (Stevens et al., 2007) including fishes (Whiteley et al., 2009; Morrongiello et al., 2010; Clarke & Schluter, 2011; Hancock et al., 2013; Stevens et al., 2014). A camera’s response to light can be highly dynamic. Therefore, before each session, it was important to test the camera’s light meter and white balance. We chose to film (rather than photograph) the fish because filming allowed us to (i) more easily capture a free swimming fish in focus and (ii) had a higher signal to noise ratio, compared to a single shot (unpublished data). A colour-checker card (ColorChecker, X-rite Inc., Oakleigh, Victoria, Australia) and white and grey standards (Pro Photography Digital) were included in a subset of the photographs (see Whiteley et al., 2009; Morrongiello et al., 2010; Clarke & Schluter, 2011). We were then able to compare these standards (grey reflectance and white standard) within and between images. We found that the response of red, green and blue (RGB) colour channels was not appreciably different with respect to the grey standards and that any variation we did see was extremely minor (< 2% difference between images) compared to the variation that existed, both within and between fish.

Lighting was provided by three white LED (light-emitting diode) lights, one at a perpendicular angle to the test tank and the other two elevated and at a 45-degree angle. LED lights have been used successfully in the past (Svensson, 2007), as they provide a stable colour temperature and light intensity over time. A total of 23 dark individuals (i.e. fish with no gold pigmentation on the body), 20 mottled individuals (i.e. fish in the process of transitioning to gold colour, with approx. 50% gold and 50% dark pigmentation; Dickman et al., 1988, which constitute approximately 10% of the Australian population) and 27 gold individuals (i.e. fish that had completely transitioned, with no dark spots on the body) were filmed on both light and dark substrates. To do so, each fish was transferred between substrate treatments, where we repeated the acclimation period and filming procedure. We randomly assigned half of the fish to be filmed on dark substrate first followed by the light substrate; with the remainder filmed in the reverse order. The experimental tanks were filmed without fish, haphazardly before, after and between replicates (n = 20, per substrate type). This was performed so that the RGB values of the two substrate treatments could be quantified and as an additional confirmation, along with colour-checker and standard cards, that lighting conditions did not alter over time.

Analysis of digital images

A single frame from each video (RAW format) was selected where the left lateral side of the fish was in focus and imported into IMAGEJ 1.47v (Kelley et al., 2012). The sum of the RGB scores were assessed (similar to Clarke & Schluter, 2011) at six (80 pixels each) identical points on each fish, for each image: four points from the dorsal side, the top lip, upper eye, start of the dorsal fin and upper tail and two from the ventral, lower eye and at the base of the pectoral fin (Fig. 1). To assess typical brightness of the two substratum/background types, RGB scores were assessed at five (80 pixels each) identical points in 20 images.

Statistical analysis

The sum of the RGB scores were assessed at the six points on each image (Fig. 1), with each fish being recorded twice, once on dark and once on light substrate. First, to determine whether the RGB scores (averaged over the 80-pixel point size) differed between the three colour types and two substrate types, we analysed each of the six measurement points separately using a split-plot ANOVA with fish colour and substrate
type as categorical variables. Because each individual was measured on both substrate types, the identity of each individual fish was treated as a random factor. We then did a test of the main effects at each point to determine whether colour morphs differed between substrates in regard to RGB scores. Secondly, to assess how well the differently coloured individuals matched the two substrate types, we applied a series of one-way ANOVAs, each comparing the RGB scores of the following five groups: the three fish colour types and of the two substrate types. Each of the six measurement points was again analysed separately. To test which pairs of groups were significantly different from each other, we then applied post hoc unplanned pairwise Tukey’s comparisons. A lack of a significant difference between a fish colour type and substratum type would imply an accurate background matching, whereas a significant difference would suggest a mismatch. Finally, to test for differences in brightness of the two substrate types, their RGB scores were compared using a two-sample t-test. We used R 3.0.0 software (R Development Core Team) for all analyses.

Collection and experimental procedures were approved by the Animal Ethics Committee of Monash University, Australia (BSCI/2012/23) and complied with all relevant State and Federal laws.

### Results

Firstly, we found that for each of the three types of individuals, there was a significant interaction between substrate type and the brightness of body colour at each point on the dorsal side of the body (Table 1), indicating that body brightness changed on different coloured substrates. By contrast, there was no significant interaction on the ventral side of the body, at the lower eye, and pectoral fin points (Table 1). We found that dark individuals became significantly lighter on light substrate compared to dark substrate at all points taken from the dorsal side of the fish (Fig. 2). Conversely gold

<table>
<thead>
<tr>
<th>Point</th>
<th>d.f.</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip</td>
<td>2</td>
<td>12.26</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Upper eye</td>
<td>2</td>
<td>14.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Dorsal fin</td>
<td>2</td>
<td>19.06</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Tail</td>
<td>2</td>
<td>22.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lower eye</td>
<td>2</td>
<td>2.69</td>
<td>0.07</td>
</tr>
<tr>
<td>Pectoral fin</td>
<td>2</td>
<td>0.84</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 1 Output of split-plot ANOVAs, investigating interactions between substrate type and fish colour morph.

![Fig. 2](https://example.com/f2.png) RGB scores taken at six identical points on each fish on two different coloured substrates, dark and light. (a) top lip, (b) upper eye, (c) start of the dorsal fin, (d) tail fin, (e) top of the pectoral fin, and (f) below the eye. Lower RGB scores indicate darker colour.
and mottled individuals showed the opposite relationship, becoming darker on light substrates, compared to on dark substrates, except for the dorsal fin point of mottled morphs where there was no significant difference (Table 2).

On the ventral side of the body, dark individuals at the pectoral fin point did not differ significantly from light and dark substrates, gold individuals did significantly become darker on light substrate and lighter on dark substrate, mottled individuals did not differ significantly from light and dark substrates (Fig. 2). At the lower eye point on the ventral side dark individuals did differ significantly from light and dark substrates, gold individuals became darker on light substrate and lighter on dark substrate, mottled individuals did not differ from light and dark substrates (Table 2).

Secondly, pairwise comparisons revealed that on dark substrate, dark individuals did not significantly differ from their background on the dorsal side, except for the dorsal fin, but did differ from the background on the ventral side of the body (Table 3). By contrast, gold and mottled individuals significantly differed from both the dark and light substrates on all of the measured parts of the body (Table 3).

Finally, we found that the dark substrate was significantly darker than the light substrate (Mean ± SE; RGB score of dark substrate = 48.1 ± 3.6, light substrate = 143.3 ± 3.5; Two-sample t-test, $t_{86} = 18.91$, $P = 0.0001$).

### Table 2
Output of main effects test at each point to determine whether colour morphs differed between substrates in regard to RGB scores.

<table>
<thead>
<tr>
<th>Point</th>
<th>Gold morph</th>
<th>Dark morph</th>
<th>Mottled morph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f.</td>
<td>t-value</td>
<td>P-value</td>
</tr>
<tr>
<td>Lip</td>
<td>134</td>
<td>−2.58</td>
<td>0.01</td>
</tr>
<tr>
<td>Upper eye</td>
<td>134</td>
<td>−2.78</td>
<td>0.006</td>
</tr>
<tr>
<td>Dorsal fin</td>
<td>134</td>
<td>4.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tail</td>
<td>134</td>
<td>−3.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lower eye</td>
<td>134</td>
<td>−2.78</td>
<td>0.006</td>
</tr>
<tr>
<td>Pectoral fin</td>
<td>134</td>
<td>0.29</td>
<td>0.76</td>
</tr>
</tbody>
</table>

### Table 3
Output of one-way ANOVA with an unplanned pairwise comparison comparing RGB scores from colour morphs to dark substrate RGB scores and to light substrate RGB scores.

<table>
<thead>
<tr>
<th>Point</th>
<th>Gold morph</th>
<th>Dark morph</th>
<th>Mottled morph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light substrate</td>
<td>Dark substrate</td>
<td>Light substrate</td>
</tr>
<tr>
<td></td>
<td>t-value</td>
<td>P-value</td>
<td>t-value</td>
</tr>
<tr>
<td>Lip</td>
<td>219</td>
<td>10.39</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Upper eye</td>
<td>219</td>
<td>10.21</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Dorsal fin</td>
<td>219</td>
<td>11.44</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Tail</td>
<td>219</td>
<td>10.57</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Lower eye</td>
<td>219</td>
<td>6.89</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Pectoral fin</td>
<td>219</td>
<td>7.02</td>
<td>&lt;0.000001</td>
</tr>
</tbody>
</table>

### Discussion
In this study, we investigated the background matching ability of the colour polymorphic cichlid, *A. labiatus*. We found a difference in the background matching ability of the different colour morphs. Dark individuals, which are more abundant in natural populations, significantly altered the shade of their body colour between substrates, being lighter on light coloured substrate and darker on dark substrate. Gold individuals, less common in natural populations, along with transitioning (mottled) individuals, did not alter their body to match their background substrate. Instead, gold and mottled individuals tended to become darker on light substrate and lighter on dark substrate.

*Amphilophus* cichlids are common and widespread in Nicaragua and individuals regularly traverse between dark volcanic rocks to lighter sand and silt habitats, in both the two large ‘great’ lakes (Lake Nicaragua and Lake Managua) and the smaller and more numerous crater lakes (Barlow & Munsey, 1976; Cole, 1976). A strategy for avoiding predators across habitats could involve colour matching against a range of backgrounds; therefore, an individual ought to express a phenotype that is optimal for camouflage in varied environments by altering its body colour (Wente & Phillips, 2003; Ruxton et al., 2004). We found that dark-coloured individuals altered the brightness of their body colour to correspond to the substrate they were
on, which was first suggested (but not formally investigated) by Barlow (1976). We found that, quantitatively, background matching of the dark morph was not perfect. Instead dark individuals may be employing a ‘compromise’ strategy, matching the specific brightness of common backgrounds and more generally of other less frequently encountered backgrounds, rather than being specialized and matching one type of background. The dark morph did not alter the dorsal and ventral sides of the body equally, with the ventral side of the body generally always being lighter and having a less dramatic change across substrate types. This pattern is consistent with counter shading, a noted camouflage strategy, which individuals employ to reduce the chance of detection by predators or prey (Thayer, 1896; Stevens & Merilaita, 2009). To be effective, a camouflage or background matching pattern needs to be a representation or approximation of a sample of the backgrounds normally viewed by predators (Endler, 1978; Merilaita et al., 1999; Houston et al., 2007). This may be an efficient strategy for dark individuals, allowing them to camouflage against a range of backgrounds as they move through a heterogeneous environment (Barlow, 1976; Stevens & Merilaita, 2009).

As hypothesised, we did not observe gold individuals (or transitioning mottled individuals) altering the shade of their body colour in a manner that would have matched background substrates. Instead, gold individuals appeared to darken their body on light substrate (background) compared to on dark substrate. This result was unexpected, especially due to the general conspicuousness of gold individuals. Regardless, it appears that gold individuals do not have the same cryptic ability as dark individuals and are, in general, more conspicuous. Being more visually conspicuous has been shown to increase predation risk and, importantly, has been shown to influence colour morph distributions in other species of fish (Endler, 1978, 1980; Godin & McDonough, 2003; Young et al., 2011).

Disparity in predation rate between colour morphs is considered to be important in contributing to the maintenance of stable polymorph frequencies (Losey et al., 1997). The results from our study show that in *A. labiatus*, individuals of the dark morph are able to plastically adjust the shade of their body colour in response to different substrate colour much more effectively than the gold morph. Indeed, evidence suggests that differences in background matching ability may be present in dark and gold individuals even before the transition process has begun, with Dickman et al. (1990) reporting that genetically gold *Amphilophus* juveniles have poorer control over their body colour and markings than genetically dark individuals.

The lakes of Nicaragua contain several large predators capable of consuming small to mid sized *Amphilophus* cichlids, including piscivorous fish, birds and caimans (Davies, 1976; Blake, 1977). Difference in predation pressure in *Amphilophus* cichlid morphs has been suggested previously (Annett, 1986, 1989; Dickman et al., 1990) and do not appear to be due to any behavioural differences in antipredator response. More recently, conspicuousness of gold individuals to both fish and bird predators has also been demonstrated experimentally (Kusche & Meyer, 2014; Torres-Dowdall et al., 2014). It is important to bear in mind, however, that the susceptibility of dark and gold individuals to different predators is likely to depend on a range of factors, such as the predator’s visual acuity, their mode of attack and the environmental conditions in which the animals occur (e.g. turbidity) (Stuart-Fox et al., 2006). Hence, more work is needed to understand how differences in colouration might influence susceptibility of dark and gold individuals to different predators and under different environmental conditions.

More generally, the maintenance of a stable colour polymorphism is likely to be the result of complex interactions, including behavioural and physiological differences, with our results suggesting that one such difference is a background matching disadvantage of individuals of the gold morph. These behavioural and physiological traits may play a role in maintaining colour polymorph frequencies, via correlated selection, compensating for colour morph disadvantages in certain circumstances (Sinervo et al., 2001; Lark, 2002; Gray & McKinnon, 2007; Hadfield et al., 2007). For example in gouldian finches (*Erythrura gouldiae*), different colour morphs employ distinct behavioural strategies, particularly in regard to aggression, which in turn leads to differences in hormone expression and immune performance (Pryke et al., 2007). In addition, the aggressive red morph finches suffer more greatly from stress in social situations where they are not rare and females have elevated stress when they are paired with incompatible mates, that is nonlike colour morphs (Pryke et al., 2007, 2011). It is also likely that multiple mechanisms are acting to maintain the *Amphilophus* cichlid colour polymorphism. However, in the introduced Australian population, gold morphs exist at a much higher frequency than in their native range, over 60%, compared to < 20% (Elmer et al., 2010). This may be due to a founder effect and/or individuals being potentially freed from the assemblage of key predators that occur in their native habitat, although more work is needed to test these possibilities.

In summary, *A. labiatus* colour morphs differed in their background matching ability in response to different substrates. Together our results suggest that differences in the ability to background match could play a potentially important role in maintaining colour polymorphism frequencies in the wild.
Acknowledgments

We thank Fiona Kang for her help with data collection, Andreas Svensson and Kaspar Delhey for advice on background theory and methodology, Nicholas Deal for help with statistical analysis, and Rowan Jacques-Hamilton and members of the Behavioural and Evolutionary Ecology Research Group for their assistance with animal husbandry. We thank two anonymous reviewers for improving the manuscript. This research was supported by grants from the Holsworth Wildlife Research Endowment and the Linnean Society of New South Wales (to WS).

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Data deposited at Dryad: doi:10.5061/dryad.r8s59

Received 2 September 2014; revised 4 December 2014; accepted 10 December 2014