



Research

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Artificial selection for food colour preferences

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Colour is an important factor in food detection and acquisition by animals using visually based foraging. Colour can be used to identify the suitability of a food source or improve the efficiency of food detection, and can even be linked to mate choice. Food colour preferences are known to exist, but whether these preferences are heritable and how these preferences evolve is unknown. Using the freshwater fish *Poecilia reticulata*, we artificially selected for chase behaviour towards two different-coloured moving stimuli: red and blue spots. A response to selection was only seen for chase behaviours towards the red, with realized heritabilities ranging from 0.25 to 0.30. Despite intense selection, no significant chase response was recorded for the blue-selected lines. This lack of response may be due to the motion-detection mechanism in the guppy visual system and may have novel implications for the evolvability of responses to colour-related signals. The behavioural response to several colours after five generations of selection suggests that the colour opponency system of the fish may regulate the response to selection.

1. Introduction

Evaluating the visual appearance of potential food is important for accurate detection and acquisition in visually based foraging. Consequently, many animals have developed highly stereotyped food preferences. For example, bees have consistent preferences for objects that contain symmetrically radial patterns (an efficient way to recognize flowers) [1], flower colour facilitates learning of foraging behaviour in bees [2], butterflies [3] and birds [4,5], and innate colour preferences allow an efficient harvest of local nectar in the bumblebee *Bombus terrestris* [6]. Preferences for coloured food items may also have shaped visual sensitivities in primates, where the maintenance of trichromatic vision is thought to be linked to food detection [7–10].

Despite the importance of food colour preferences to many aspects of behavioural and evolutionary ecology [11–13], the heritability of these preferences is unknown. Many foraging preferences are thought to be flexible towards unpredictable environments and variability in food reward [13]; however, this may be a risky option when foraging attracts costs. Innate preferences may represent a safer and more efficient method of food detection, provided that the environment is predictable enough for heritable rules to work. Colour preferences are known in a wide variety of species, and can affect food choice [11,14], but how these preferences evolve is largely unknown and untested.

One such species that has distinct food colour preferences is the guppy, *Poecilia reticulata*. Food colour preferences in this species are thought to be of particular importance as they may have been co-opted into mate choice [15]. A study by Rodd *et al.* [15] showed that guppies have a food colour preference for orange. This matches a female mate preference for the same colour [16,17], indicating that mate preferences may have co-evolved with food colour preferences; males may have exploited a pre-existing sensory bias in the visual system that evolved as a response to food detection or recognition. However, the heritability of this food colour preference, an essential assumption of this hypothesis, has never been investigated. In fact, to the best of our knowledge, the heritability of food colour preferences, in general, has never been tested.

Using guppies as a model system, we conducted artificial selection to investigate whether these food colour preferences are heritable and to test how quickly any evolutionary response is likely to occur. Guppies live in continuously flowing freshwater streams, and are omnivorous, opportunistic foragers that feed on insect larvae, small invertebrates, fruit and algae [18–20]. Because benthic vegetation is often absent in such streams, and food items tend to move with the current of the water, we used moving-simulated prey items to conduct this study. We used two different colours, red and blue, to select on the opposite ends of the guppy spectral sensitivity range (300–700 nm [21]). Because food colour may be important in mate choice in this species [15], we predicted that both food colour preferences will be heritable. We also wanted to provide further evidence as to whether co-option is likely to have led to known female preferences in this species.

2. Material and methods

(a) Husbandry

Guppies used for the experimental populations were first to second generation wild-caught fish from Alligator Creek, Queensland, Australia (19°26.79' S 146°58.65' E). Fish were maintained at a $24 \pm 1^\circ\text{C}$ and 12 h light/dark regime with brown and green flake food provided daily. Individuals were housed in large 194 l mixed-sex glass tanks containing around 150 juveniles and adults of both sexes (sex ratio approx. 1 : 1) prior to use in the experiments.

(b) Visual stimuli

In order to create a selectable proxy for foraging behaviour, visual stimuli from red and blue laser pointers (wavelengths of 650 and 450 nm, respectively) were used to create slowly moving spots inside a black-walled wooden box measuring 48 cm (height) \times 40 cm (depth) \times 32 cm (width) containing a removable 6 l tank with a sand substrate (see electronic supplementary material, figure S1). We named this apparatus the 'maculator' (literally, spot generator, from the Latin 'macula', meaning spot). These wavelengths were chosen so that selection acted on very different guppy cones; guppies have peak visual sensitivities at 359, 408, 464, 533, 543 and 572 nm (see electronic supplementary material, figure S2) [22–24]. Both the red and blue spots were approximately 2 mm in diameter when measured on the floor of the tank containing sand and water—about $10\times$ larger than the minimum spatial resolution of guppies when seen from 2 cm [25].

On the internal ceiling of the maculator, at a height of 37 cm, a motor (later referred to as the 'spot motor') turns a mirror-covered octagonal cylinder. When a mirror is illuminated by a laser, a single spot is projected onto and moves in a straight line across the tank floor below. The motor was set at a constant speed of 12 revolutions a minute, so that the spot moved across the sand floor at about 16 cm s^{-1} . The moving spots simulated food items being carried at a speed similar to that of a medium-flowing stream (the type of habitat in which a guppy would be found in the wild). Guppies will chase a number of moving prey items, including brine shrimp, daphnia and their own offspring.

The irradiance spectra of the spots and the ambient light in the chamber were measured using a calibrated Li-Cor photometer (LI-189) placed on the bottom of the tank. The radiance of the spots reflected off the sand was measured with an Ocean Optics USB2000 + spectrometer connected to a calibrated sensor aimed at the spot from 2 cm away. The ambient light inside the maculator ($6.6\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$) was created by a 15 watt incandescent bulb placed at 25 cm above the floor of the apparatus—within the natural range in the wild [26]. The spot light intensity on the sand

was standardized with a neutral density wheel for each laser colour to equal total irradiance ($2.8\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$) for both spots, so that only colour and not luminance was different between the treatments. The resulting radiance off the sand was 0.4 and $0.6\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}\text{ str}^{-1}$ for the blue and red spots, respectively, owing to the tan sand colour. We calculated the perceived luminance of the two laser spots, based upon the guppy double cone guppy visual parameters (optical media provided by Dr Ron Douglas; cone peak sensitivities from [22–24]) and the radiance spectra of the laser spots (see [17,27–29]). Luminance (sometimes known as brightness) represents the total light intensity as measured by the cone types responsible for detecting luminance contrast (the double cones). Based on these calculations, the perceived luminance for the blue spot was 1.06 times that of the red. The reduced difference in luminance compared with total radiance is due to the incandescent adapting light; it is more intense in long wavelengths, so chromatic adaptation makes the cones less sensitive to red than blue light. Chromatic adaptation was allowed for in the von Kries correction (see [29]). We calculated the number of just noticeable differences (JNDs) between the two stimuli to determine whether the observed difference in perceived luminance would be apparent to the fish (see [29,30]). This resulted in a JND of 0.01, well below the threshold of 1, indicating that no difference in luminance could be detected by the fish. The spectra for the double cones and laser spots used in the calculations are given in the electronic supplementary material, figure S3.

(c) Recording foraging behaviour

In order to record foraging behaviour, individual fish were placed into a 6 l tank that had a sand substrate and black mesh around the sides. The sand substrate enabled the observer to identify fish behaviour easily owing to high visual contrast, and the black mesh was used to minimize spot reflection from the tank walls. The tank was filled to a depth of 6 cm above the substrate with water that had been taken from their mixed-sex maturation tank to assist acclimation. Fish were allowed to acclimate for 6 min in these tanks prior to the fish and tank being placed into the maculator; this allowed fish to return to normal behaviour after being moved. Fish then had a further 2 min to both behaviourally and chromatically acclimate inside the apparatus. During this time, the spot motor was running, but the mirror was not illuminated, ensuring fish were acclimated to vibrations and noise created by the motor. After 2 min, the mirror was illuminated by either a red or blue light beam from the laser pointer, creating a single moving coloured spot on the floor of the tank. The octagonal mirror ensured that once the first spot had travelled the floor of the tank another spot would follow; this continued until the end of the trial. Thirty-six consecutive spots were seen in each trial. Those fish that chased the spot continued to do so despite no food reward being given. The observation session commenced when the first spot appeared. A video monitor connected to a camera inside the maculator allowed the observer to watch behaviour, so that there were no disturbances to the fish during each session.

Fish were observed for 3 min and their behaviour recorded. The number of chases of the moving light spot (c), the number of orientations towards the spot (o) and the latency until the start of the first behaviour (either chase or orientation; l) were combined into a total score $T = (3c + o)/l$. T is higher when a fish shows any or all of the chase-related behaviours. For example, a fish that chased after 2 s of the trial commencing and that carried out four chases and two orientations during the trial would receive a score of $((4 \times 3) + 2)/2 = 7$. A fish that orientated after 50 s of the trial commencing with no further behaviours would receive a score of $((0 \times 3) + 1)/50 = 0.02$. Fish that did not chase or orientate towards the spot received a latency score of 180, which

resulted in an overall score of 0. Chases were weighted three times more than orientations because they were the most obvious indicator of the simulated foraging behaviour. The number of nips was also measured, but this was not used in the selection criteria because it was initially relatively rare, particularly for blue spot stimuli (in generation 0, the mean number of nips towards the blue and red spots was 0.013 and 0.11, respectively).

(d) Test of foraging behaviour

We verified that the blue and red light spots simulated foraging behaviour by testing two groups of fish with a high and low motivation for foraging and observing their behaviour inside the maculator. To create the treatment groups, males and females were selected at random from the stock tanks and within-sex size matched within 2 mm. These fish were then divided into two treatments: fed and relatively food-deprived. Fish in the fed treatment received floating flaked fish food once daily as normal, whereas fish in the food-deprived treatment received food on alternate days. Food-deprived fish were still able to forage on microflora and fauna on the gravel of their tanks (most of their natural food); this ensured that the fish were never in danger of actually being starved. Within these food treatments, the fish were separated into two further treatments corresponding to spot colour. This resulted in four treatments—red hungry (RH), red fed (RF), blue hungry (BH) and blue fed (BF)—with 16 fish of each sex in each treatment (128 fish in total).

Fish were housed in 6 l tanks in single sex groups of four according to food and colour treatment. Sexes were separated to control for energy expended from mating effort and female conditioning to colours seen in males. To control for differences in behaviour over time and to ensure all fish had the same treatment time (not all fish could be measured in one day), four tanks for each treatment were set up on alternate days for 4 days. For example, on the first day, two BH and two RH tanks were set up with four males and four females each, the second day BF and RF, and so on. After 4 days of treatment, fish were tested individually in the maculator with the spot colour determined by the designated treatment (R or B). Differences in the chase behaviour of fish in the four treatments were analysed using Bonferroni-corrected Mann–Whitney *U*-tests in the program R [31], because the data were not normally distributed.

(e) Experimental population

We conducted artificial selection experiments to investigate whether chase behaviour for red or blue spots was heritable and to estimate its magnitude. Up to eight juveniles (four males and four females when family size allowed) from 180 different females were used to initiate the experimental population, representing a compromise between increased effective population size and brood size. Siblings were split, by sex, into one of six spot colour treatments: blue (B), red (R) or control (C), each with two replicates, blue (B1, B2), red (R1, R2) and control (C1, C2). A total of 1200 individuals made up the initial experimental population, with 200 individuals (100 males and 100 females) assigned to each of the six treatments. In order to maximize artificial selection efficiency, males and females were housed separately to ensure all females were virgins after artificial selection in each generation; guppies have sperm storage [32].

(f) Artificial selection

After maturation (approx. 20 weeks), fish were placed in the maculator, and their tendency to chase the moving light spot was observed and recorded. A chase score (*T*) was obtained for each individual. R replicates were scored with moving red spots and B replicates were scored with moving blue spots. C replicate fish were scored for both red and blue spot chasing,

and the order in which they encountered the two colours was alternated to control for any order effects. These are referred to as CB1 and CR1 for the first C replicate, and CB2 and CR2 for the second; CB1 and CR1 represent the fish in C1, CB2 and CR2 represent the fish in C2.

The chase score *T* was determined for 200 fish (100 of each sex) within each replicate of the three selection lines (R, B, C) in the first selection (generation 0) and 160 (80 of each sex) in the following four generations (1–4). This represents a compromise between time to measure and effective population size. A total of 5040 fish were measured, and 6720 scores were recorded, because control fish were measured twice in order to obtain both their red and blue scores.

Once all fish had been scored within a given treatment, the 40 males and 40 females with the best *T* in their replicate were placed into a 194 l glass aquarium and allowed to mate for two weeks. Fish in the control lines were selected at random and 40 of each sex collected from the population allowed to mate in the same manner. After two weeks of mating opportunity, females were removed and housed in individual 6 l tanks containing 5 mm plastic mesh (to protect offspring from cannibalism) until the females gave birth. Males were moved to a new 54 l home tank according to their treatment.

Once a female gave birth, she was removed from her tank and her fry were left to mature. Sexes were separated prior to maturation. Up to four female and four male juveniles (depending upon family size) were taken from each family and placed, according to treatment, into 54 l glass aquaria to mature. Sexes were again housed separately. Males were housed with both virgin and non-virgin females from stock tanks in order to maintain normal mating behaviour. After approximately 20 weeks, these new generation fish were scored using the maculator and the process repeated. *T* scores from five generations were obtained (generations 0–4). Offspring mortality rates, indicative of the level of inbreeding, were recorded for each generation. Offspring mortality rose from 5.2% and 6.9% in generations 1 and 2, respectively, and to 28.8% and 27.2% in generations 3 and 4, respectively.

(g) Calculation of heritabilities

Realized heritabilities (h^2) were calculated for each selection line using the slope coefficient of the regression of the response to selection for increased *T*, against cumulative selection differential [33]. Realized heritabilities were also calculated for control-corrected selection lines; control correction was performed by subtracting the mean of both control replicates from the individual values of the selected line, using red controls for R lines and blue controls for B lines.

(h) Mechanistic test of visual stimuli

To test possible visual mechanisms by which different chase behaviours evolved, we tested the final generation (5; the offspring of generation 4) with a green spot (532 nm) in addition to the blue and red spots we used in the previous generations. Between 10 and 20 fish (owing to time and fish inbreeding constraints) of each sex from each line (B1, B2, R1, R2, C1 and C2) were selected randomly and assigned to one of the three spot colours. In order to avoid possible habituation to the moving spot stimulus, test fish were only exposed to one spot colour treatment. The chase score *T* was recorded in the same way as before. Mann–Whitney *U*-tests were used to identify significant differences between the treatments. Control correction was again performed by subtracting the mean of both control replicates from the individual values of the selected line, using red controls for R lines and blue controls for B lines.

The perceived luminance of the green laser spot on reflection from the sand was 5.5 times that of the red spot and 5.2 times that of the blue spot, owing to the sand colour. This resulted in

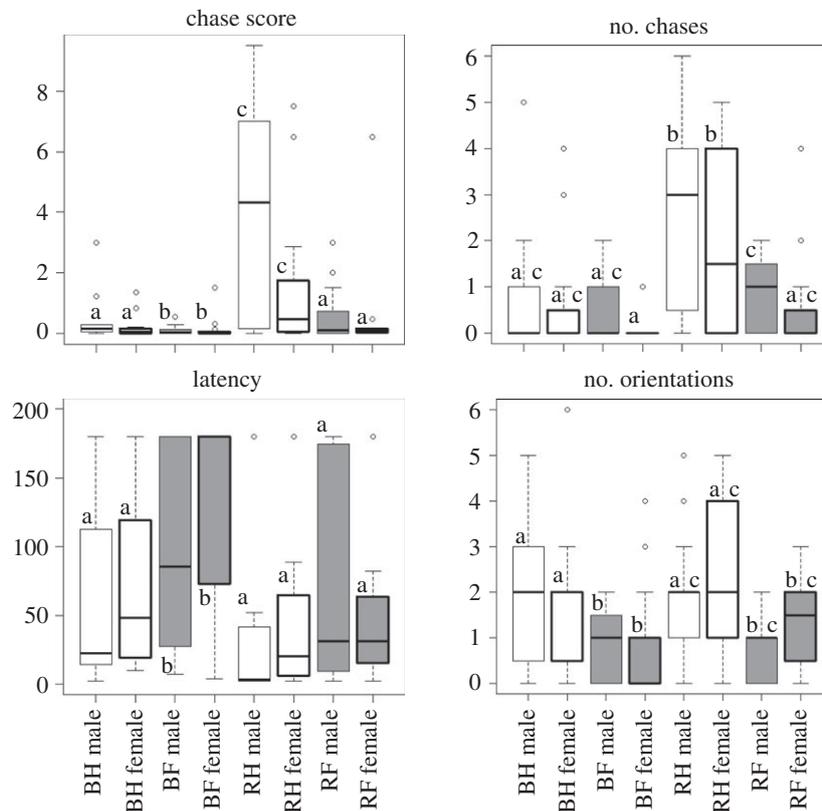


Figure 1. Box plots showing the median (horizontal line), upper and lower quartiles (box length), and range of the effect of feeding treatment on the selection criteria: chase score (T), number of chases (c), number of orientations (o) and latency (l). Whiskers show the lowest and highest values within 1.5 times of the interquartile range. White boxes represent hungry fish and shaded boxes represent fed fish. Thinner outlines represent the blue treatment and thicker outlines represent the red treatment. $n = 32$ for each of the treatment combinations (128 fish were tested in total). Fish that did not chase or orientate towards the spot received a latency score (l) of 180 and a T of 0. Different letters denote significant differences (at $p < 0.05$) between treatments with the Bonferroni correction.

JNDs of 0.74 and 0.73, respectively. This is again below the threshold of 1, indicating that the fish would not perceive this difference in luminance. The radiance of the green laser on reflection from the sand was $0.08 \mu\text{mol photons m}^{-2} \text{s}^{-1} \text{str}^{-1}$.

3. Results

(a) Test of feeding behaviour

Within treatments, both red and blue food-deprived fish (RH, BH) exhibited more spot pursuit behaviour than fed fish (RF, BF), indicating that our selection criteria were related to foraging behaviour (figure 1). Chase scores (T) were significantly higher in both RH and BH trials than in the BF and RF trials (Mann–Whitney U -tests: RH versus RF, $W = 733.5$, $p = 0.018$; BH versus BF, $W = 723.5$, $p = 0.023$). RH fish exhibited more chases than their RF counterparts (Mann–Whitney U -tests: chases, $W = 752$, $p = 0.0041$), but latency to chase and the number of orientations were not significantly different (orientations, $W = 680.5$, $p = 0.12$; latency, $W = 653$, $p = 0.35$). BH fish exhibited a higher number of orientations and a lower latency to chase (orientations, $W = 773$, $p = 0.0018$; latency, $W = 707.5$, $p = 0.045$) than BF fish. No significant difference was observed between sexes ($W < 145$, $p > 0.33$). All comparisons were Holm–Bonferroni-corrected [34].

Across treatments, fish in the red treatments scored a significantly higher T (food-deprived, $W = 717$, $p = 0.0023$; fed, $W = 711.5$, $p = 0.0065$) than the blue treatment.

(b) Artificial selection

Artificial selection will reveal additive genetic variation if present. A response to artificial selection for increased T was seen immediately in the two red lines R1 and R2 (figure 2). Although an initial increase in T was seen between generation 0 and generation 1 in the blue lines (B1,B2), no increase was observed after this. No significant response was observed in the control lines C1 and C2, although the mean T fluctuated between 0.58 and 0.9. The highest mean T was seen at generation 1 for both B1 and B2, and generation 3 for both R1 and R2. The subsequent decrease in the R chase scores after generation 3 may have been due to the highly elevated mortalities. Table 1 shows the realized heritabilities both with and without standardization by the controls. The variation in scores in the R lines was notably higher than that of the B lines. Consistent with the food treatment experiment, no significant difference was observed between the sexes, again indicating that this is not a sex-linked behaviour and is therefore unlikely to be due to sexual preferences.

(c) Test of possible visual mechanisms

The chase score (T) was recorded for three different spot colours (blue, red and green) for each of the treatments at the final generation (5; the offspring of generation 4) to test whether there was a correlated response between colours. Figure 3 shows the results for the treatment replicates combined (note that CR1/CB1 and CR2/CB2 are actually C1 and C2, respectively; the mean of these is represented by

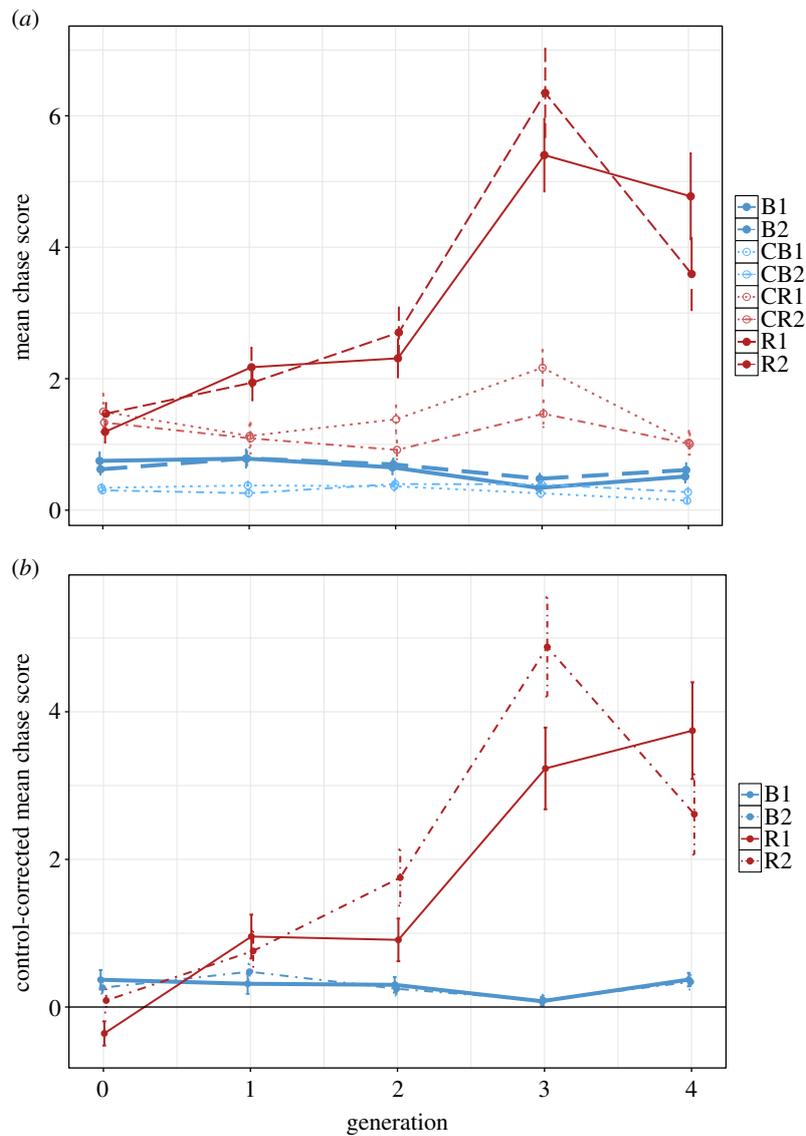


Figure 2. Mean chase scores (T) per generation with standard errors. (a) Mean scores and (b) control-corrected mean scores (experimental values – control values). R1 and R2 are the red-selected lines, B1 and B2 are the blue-selected lines, and CR1, CR2, CB1 and CB2 are red and blue control lines, respectively. Although the same fish were measured for both the red and blue control lines, these are shown separately on the graph as CB1 and CR1, and CB2 and CR2. (Online version in colour.)

Table 1. Tables showing realized heritabilities (h^2) and their standard errors in parentheses, for generations zero to four, for each selection line, before control correction and after control correction. Asterisks denotes significance at $*p < 0.05$ and $**p < 0.01$.

	B1	B2	R1	R2
before control correction	0.096 (0.137)	0.081 (0.075)	0.298 (0.084)*	0.23 (0.068)
after control correction	0.030 (0.098)	0.070 (0.092)	0.304 (0.049)**	0.249 (0.116)

the control line in figure 3). Control-corrected scores were calculated by subtracting the mean of the C lines from the mean of the R and B lines. There was no difference in chase score between the sexes ($p > 0.09$) nor between the treatment replicates ($p > 0.06$) within colours.

The general trend was for both R and B selection lines to have a significantly reduced response to the colours for which they were not selected, relative to the C lines. When control-corrected, the B-selected lines had the highest T towards the blue (450 nm) spot, but lower T towards the green (532 nm) and red (650 nm) spots. The R-selected lines had the highest T towards the red (650 nm) spot, but lower scores towards the blue (450 nm) and green (532 nm) spots. The C lines had the highest chase score for the green

(532 nm) spot, and intermediate chase scores towards the blue (450 nm) and red (650 nm) spots. Pair-wise Mann–Whitney U comparisons on the uncorrected data indicate that the R lines had significantly lower T towards the blue (450 nm) spot and significantly higher T towards the red (650 nm) spot than the B and C lines (table 2). The C lines also have a significantly higher T towards the green (532 nm) spot than both the B and R lines.

4. Discussion

Our study has shown that hunger-related colour preferences are heritable and can respond significantly to artificial

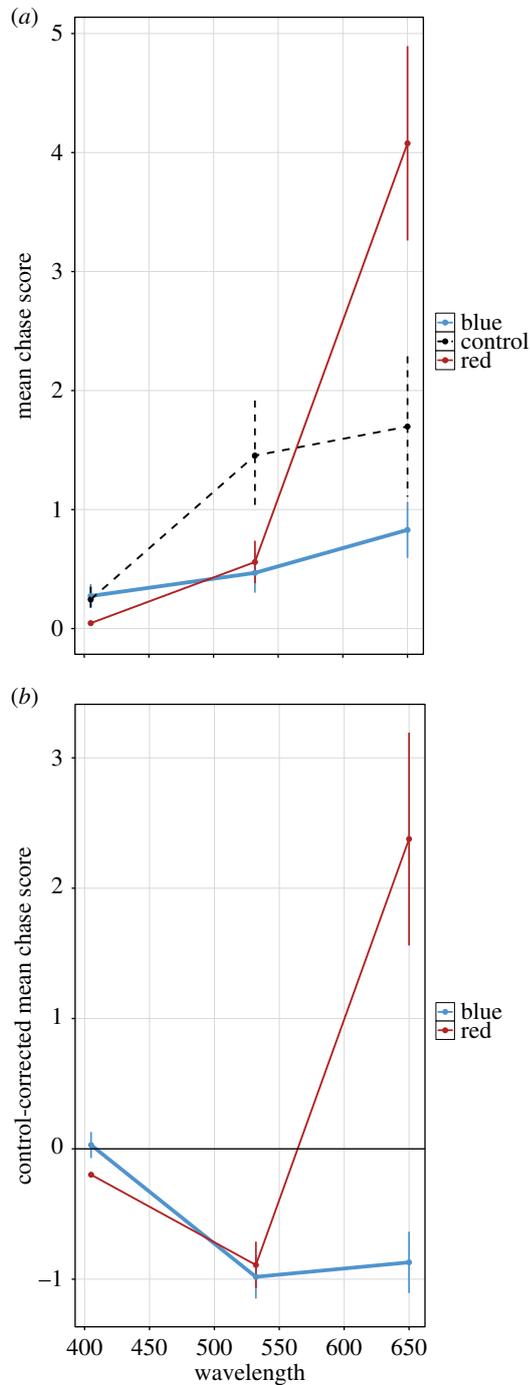


Figure 3. Mean chase scores (T) measured across three wavelengths (450 nm, 532 nm and 650 nm) for each treatment (replicates combined) with standard errors. (a) Mean score and (b) control-corrected mean score. Control-corrected scores were calculated by subtracting the mean of the C (control) lines from the mean of the R (red) and B (blue) lines. C is the dashed line, B is the solid thick line and R is the solid thin line. Fish in the C lines were selected at random and each measured twice, once for chasing the red spot (CR) and once for chasing the blue (CB) spot; the control line in the figure represents these fish. (Online version in colour.)

selection within only four generations. Surprisingly, we found a response for only one of the two selected colours, which suggests limited evolutionary potential in our blue lines based solely on differences in food colour. We found heritability (h^2) values of 0.25 (s.e. 0.12) and 0.30 (s.e. 0.05) for the red treatment replicates, and 0.03 (s.e. 0.10) and 0.07 (s.e. 0.09) for the blue ($h^2 \cong 0$ for blue). These values are lower than others reported for foraging behaviours in this

species [35], but this is despite very high levels of inbreeding and mortality in the final two generations, which would presumably have increased the effects of genetic drift. Previous studies have shown that the colour sensitivity in the guppy does respond to selection [21].

It can be difficult to determine exactly what artificial selection affects. In order to understand whether it was behaviour, motivation or vision that we were selecting, we tested the chase tendency of our final generation with three wavelengths: blue (450 nm), green (532 nm) and red (650 nm). If we were selecting on a general motivation to chase, we would be likely to see an increase in the red lines' chase scores towards the blue (450 nm) spot, and both red and blue lines should chase the green (532 nm) spot as frequently as the control lines. However, our results show a decreased tendency to chase the spot colours for which the lines were not selected. Disentangling a motivation to chase red items and an increase in the visual sensitivities for red is more difficult, although our results suggest that changes in the visual system may have occurred.

Because our stimuli were of a very narrow bandwidth (lasers), any correlated response to selection is most likely to be owing to the broad spectral sensitivity of each guppy cone. Guppies have cone types with maximum sensitivities at 359 (ultraviolet, abundance $\approx 1\%$), 408 (short wavelength violet, abundance $\approx 25\%$), 465 (short wavelength blue, abundance $\approx 35\%$) and 533/548/572 nm (medium-long wavelength, abundance $\approx 35\%$) [22,24]. Spectral sensitivity is known to respond to selection [21]. If one of our stimuli strongly stimulated a cone, then the response should evolve rapidly, but if it stimulated the cone weakly, the response should be weaker, or even non-existent if stimulation is below the cone's sensitivity threshold.

Selection on one wavelength should yield an apparent correlated response in responses to other wavelengths provided they are sensed by the same cone. Colour is coded in vertebrate (and invertebrate) visual systems by the differences between cone outputs; this is called opponency [36,37]. If the response to selection is in cone opponency, then we would expect a positive response to colours close to the favoured cone's peak sensitivity and a negative response to colours matching cones involved in opponency to the favoured cone. In the red lines, there was a strong increase in the response to red, but a decrease in responses to green and blue, compared with the controls. Similarly, the blue lines showed a significant decrease in response to green and red compared with the control, and a non-significant increase in their response to blue (figure 3). Although the opponency mechanism in guppies is not known, the pattern of positive and negative responses to selection suggests that selection may have affected the colour opponency mechanism rather than simply the cones most sensitive to the selected colour. The mechanism may lie in either the retina or brain, but opponencies are well known in vertebrate retinas, involving the horizontal and/or amacrine cells. Given this, it is simpler to propose that retinal opponency may have evolved in the selected lines.

As it is chase tendency that we have selected, it is not surprising that the red treatment lines had a strong chase tendency and response to selection. Guppies have multiple opsin genes that encode their long wavelength colour vision, which may provide broader visual sensitivity in the long wavelength range of the colour spectrum [22–24]. This visual mode may have evolved in response to high competition for orange- and red-coloured fruit on which the guppies forage; these fruits

Table 2. Results of Mann–Whitney *U*-tests of the pair-wise comparison of chase score (*T*) for selection lines towards the three light spots, red (650 nm, *n* = 140), green (532 nm, *n* = 120) and blue (450 nm, *n* = 140). Comparisons are Bonferroni-corrected.

	blue spot (450 nm)	green spot (532 nm)	red spot (650 nm)
R versus B	$W = 1493.5, p = 0.03$	$W = 804, p = 1$	$W = 587.5, p < 0.0001$
B versus C	$W = 787.5, p = 1$	$W = 498, p = 0.01$	$W = 1062, p = 0.94$
R versus C	$W = 1545, p = 0.0093$	$W = 1096, p = 0.012$	$W = 485.5, p = 0.0067$

are relatively rare and provide a favoured source of nutrients, creating high levels of competition [38]. Additionally, selection on increased chase tendency for long wavelengths has resulted in decreased chase tendency towards short wavelengths. Having a relatively increased sensitivity for long wavelengths may enable individuals to forage on food items of this colour more efficiently and would result in a higher chase tendency for the red spot over the blue; blue food items (fruit) are rarer in the wild than red or orange fruit in the rainforests above the native guppy streams. Changes in the visual system are likely to be altered through the up- and downregulation of the opsin genes [39–41]. Given the extensive complement of opsins, particularly long-wavelength-sensitive opsins in guppies [22], spectral sensitivity can easily be modified by evolved changes in opsin regulation patterns.

This sensory bias, potentially from food detection, has also been linked to female mate choice in this species [15]. Female guppies have a preference for orange coloration in males, and this is prevalent in many populations [16,42,43], but not in all [17,44]. It has been suggested that this has evolved through the visual sensitivity towards long wavelengths that probably originated from carotenoid-rich food sources such as orange fruits [15,45]. Our study helps support these findings by confirming not only a foraging preference for long wavelengths, but also the evolutionary potential for it to be selected; we have shown that there is a high heritability for foraging behaviour towards red-coloured objects. This is essential for this trait to be co-opted as a mate choice criterion.

The lack of response in the blue line is more difficult to explain. Although we might have expected the initial chase tendency of the blue lines to be lower than those of the red, because blue fruit is rare in the wild, we would still expect some behavioural and evolutionary response. Our study has shown that hungry fish had a higher chase tendency than fed fish, even for blue spots, and so we are confident that it is foraging behaviour that we are measuring. Unlike the red treatment, there is no historic association with blue food, which would make the response to selection slower. Carotenoid-based foods may be nutritionally more attractive than blue fruits and, if so, a mechanism to exploit them as a food source may have never evolved. It may also be that the reward of foraging on blue food items is highly variable, and therefore a higher degree of learning has evolved. A lack of genetic variation is a barrier to selection [46], and it is possible that this is the case in this study system, where the fish have limited behavioural variation towards the blue light.

Another explanation is that this colour has implications outside of foraging, such as for predator avoidance. If blue is linked to predators, then this could explain avoidance of blue items, particularly moving ones. This leads to interesting predictions regarding a trade-off between predator avoidance and food

acquisition; animals that are predated by species bearing a given colour should not actively forage on food of the same colour. This interaction could lead to interesting dynamics regarding two opposing naturally selected traits—foraging and predation—that have not been considered before. Unfortunately, the only natural predator having blue reflectance is the very weak predator *Aequidens pulchur*; the other natural guppy predators are much more dangerous [25] and lack blue entirely.

The best explanation for the lack of blue response is the colour sensitivity of vertebrate motion detectors. Motion detection in fishes is thought to be mediated via cones containing long-wavelength-sensitive opsins [47,48]. We selected guppies to chase moving blue spots, but if the motion-detection system is insensitive to the short-wavelength blue spots, then this means that there is no genetic variation for favouring blue moving objects, and hence no evolutionary response is possible. The fish were able to orientate towards the spot, and this may have been due to seeing the spot, but a lack of motion detection for blue would make following the spot's path difficult. The colour-sensitive nature of motion detection suggests that blues, violets and UV-based colours should not be used in sexual displays involving a lot of movement, and this prediction holds in any species where shorter wavelengths are outside the spectral sensitivity of motion-detection systems.

We have demonstrated artificial selection for foraging behaviour based solely on food colour preferences. Our results indicate that a response to selection can be elicited in a very short period of time, which may serve to allow populations to respond readily to a change in foraging conditions, such as increased water flow or high competition for food resources. We have also demonstrated that foraging behaviour towards some food colours may not be able to evolve. This may be due to a number of reasons, the most probable being the visual physiology of the fish. The relationship between colour and evolvability may affect the form of many different kinds of visual signals, and may well extend to other aspects of visual signals.

Ethics statement. The experiment was approved by the Deakin University Animal Ethics Committee, approval numbers A21-2010 and G01-2012.

Data accessibility. The raw data for this study can be found in the Dryad data repository: doi:10.5061/dryad.k6j0v.

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Author's contributions. The experiment was designed primarily by J.A.E. and the data were collected, and analysed, by G.L.C. The manuscript was prepared by G.L.C. and was revised by J.A.E.

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