Dynamic eye colour as an honest signal of aggression

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Summary

Animal eyes are some of the most widely recognisable structures in nature. Due to their salience to predators and prey, most research has focussed on how animals hide or camouflage their eyes [1]. However, across all vertebrate Classes many species actually express brightly-coloured or conspicuous eyes, suggesting they may have also evolved a signalling function. Nevertheless, perhaps due to the difficulty with experimentally manipulating eye appearance, very few species beyond humans [2] have been experimentally shown to use eyes as signals [3]. Using staged behavioural trials we show that Trinidadian guppies (*Poecilia reticulata*), which can rapidly change their iris colour, predominantly express conspicuous eye colouration when performing aggressive behaviours towards smaller conspecifics. We then show, using a novel visually-realistic robotic system to create a mismatch between signal and relative competitive ability, that eye colour is used to honestly signal aggressive motivation. Specifically, robotic 'cheats', i.e. smaller and thus less-competitive robotic fish that displayed aggressive eye colouration when defending a food patch, attracted greater food competition from larger real fish. Our study suggests that eye colour may be an under-appreciated aspect of signalling in animals, shows the utility of our new biomimetic robotic system for investigations in animal behaviour, and provides rare experimental evidence that socially-mediated costs towards low-quality individuals may maintain the honesty of dynamic colour signals.

Main Text

Many species are extremely attuned to eyes and eye-like stimuli, particularly in predatory contexts [1], and this sensitivity may predispose eyes and their surrounding tissue to evolve a signalling function towards conspecifics. Like many fish [4], guppies can rapidly (<3 seconds) change their iris colour from silver to black, which increases the conspicuousness of their eyes and has been suggested to act as a signal of aggression [5, 6]. Signals are traits that have evolved to modify the behaviour of a receiver (benefiting both sender and recipient), and stable signalling systems require costs to maintain honesty and stop exploitation from cheats [7]. Dynamic signals, such as those involving rapid colour change, are presumed to be physiologically cheap to produce, and the ability to dynamically display the signal during functional contexts also means it need not necessarily incur environmental costs such as increased predation [8]. Whilst theoretical studies on the evolution and maintenance of dynamic signals suggest socially-mediated costs play an important role in maintaining honesty, with low-quality individuals being incapable of sustainably receiving social costs from more competitive individuals [7], empirical evidence is rare [8, 9].

Using guppies, we experimentally tested whether eye colouration is used to honestly signal aggressive motivation. To initially confirm that eye colour change is associated with aggressive interactions in our study populations, we recorded iris colouration during staged food-competition trials within trios of same-sex fish caught from four populations differing in primary productivity in the Northern Range mountains of Trinidad (details in Table S1). Across all populations, we found that fish expressing black irises, and larger fish, were more likely to perform aggressive behaviours than receive them (Binomial GLMM: eye colour in females: odds ratio (OR) = 201.7, $\chi^2 = 51.36$, $p < 0.001$; males: OR = 25.8, $\chi^2 = 20.82$, $p < 0.001$; Figure 1a; body size in females: OR = 2.7, $\chi^2 = 105.77$, $p < 0.001$; males: OR = 3.4, $\chi^2 = 30.00$, $p < 0.001$; Figure 1b).

Determining whether a dynamic trait is a signal requires assessing a receiver's response once the putative trait has been experimentally disentangled from its correlated behaviours [4]. The difficulty of experimentally manipulating eye appearance in live animals may therefore explain why few studies have focused on the potential signalling function of this organ. To overcome this challenge, we designed a biomimetic robotic system that utilised a novel method of creating three-dimensional model guppies with visually-realistic colouration and patterns based on calibrated photographs (Figure 1c; see Supplemental Information for robotic methodology). This allowed us to create biomimetic robots with a mismatch between eye colouration and competitive ability (body size) to determine whether eye colouration is a functional and honest signal. In theory, our colour-calibration methods can be used to match biomimetic models to the non-UV colour vision of most animals with a modelled visual system.

We ran a binary-choice experiment where separate pairs of live female guppies were introduced to an arena with a pair of otherwise identical black-iris and silver-iris robotic female guppies that were defending separate food-patches (simulating food 'monopolisation' behaviour displayed by guppies [6], Figure S1). We found that food-patches defended by black-iris robots attracted disproportionate foraging competition from guppies larger than the robots, particularly those expressing black irises themselves (i.e. signalling aggressive motivation). In contrast, smaller guppies fed predominantly from the silver-iris robots

(Binomial GLMM, body size difference x proportion time spent with black-irises: $\chi^2 = 4.74$, $p = 0.029$; Figure 1c). If expressing black iris signals an individual's aggressive motivation, it may reliably indicate the location of a valuable, defended food resource. Honesty in iris-colour expression may therefore be partially enforced by socially-mediated costs whereby fish that dishonestly express black irises attract more competitive conspecifics and subsequently suffer increased food competition. Given that performing an aggressive behaviour is strongly predicted by the relative body size of the combatants, larger fish (particularly those more motivated to fight, as indicated by expressing black irises) that are better able to competitively feed and/or sustain costs of potential escalated fighting may therefore be attracted to smaller black-iris conspecifics. Taken together, these experiments suggest that the expression of black iris in an agonistic context is an honest signal of aggressive motivation in guppies.

Why might guppies, and possibly other fish, use their eyes to signal aggression? We speculate that blackening the iris enlarges the perceived size of the pupil, and therefore eye colour change could have initially evolved to increase the perceived size of conspecifics or exploit eye-based predator recognition cues [10], subsequently being modified into a signal. Manipulation studies within a phylogenetic framework investigating whether coloured irises exploit pre-existing biases during various behavioural contexts might be particularly revealing as to the original mechanism that has driven the evolution of this trait in guppies and other species.

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Figure

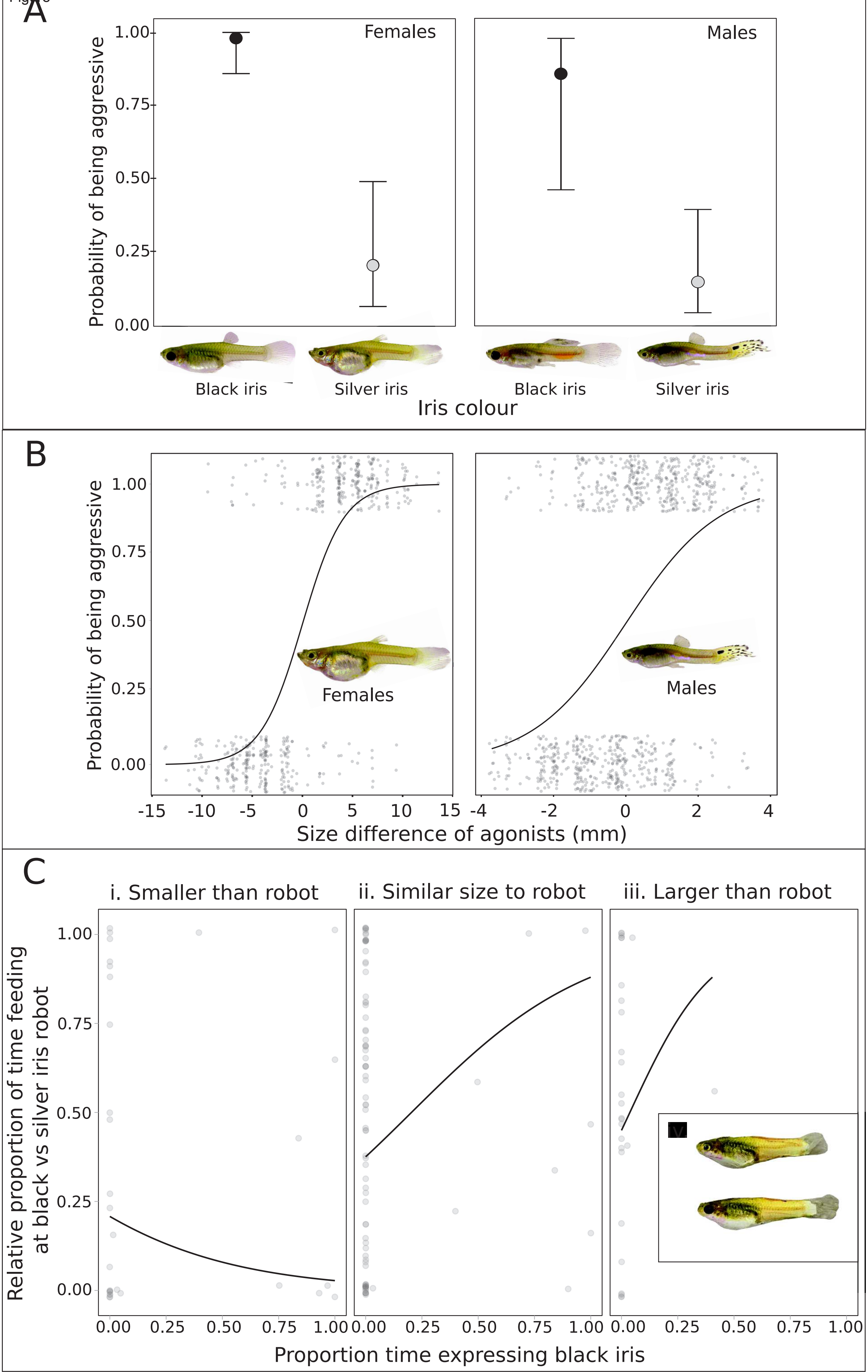
A
Probability of being aggressive
1.00
0.75
0.50
0.25
0.00
Females
Males
Black iris
Silver iris
Black iris
Silver iris
Iris colour
B
Probability of being aggressive
1.00
0.75
0.50
0.25
0.00
Females
Males
-15
-10
-5
0
5
10
15
-4
-2
0
2
4
Size difference of agonists (mm)
C
i. Smaller than robot
ii. Similar size to robot
iii. Larger than robot
Relative proportion of time feeding at black vs silver iris robot
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
Proportion time expressing black iris

Figure 1. Effect of iris colour and body size on signaller and receiver behaviours in Trinidadian guppies.

A) Iris colour predicts the probability of performing (versus receiving) an aggressive behaviour during agonistic interactions in female and male guppies. Circles are the mean within each treatment and error bars denote the +/- standard errors, both determined from a binomial GLMM. B) Body size predicts the likelihood of performing (versus receiving) an aggressive behaviour in female and male guppies. Lines are based on the predicted probability determined from a binomial GLMM. Data points are offset off 0.00 and 1.00 to aid visual clarity. C) In the robotic experiment, the proportion of time spent feeding at the black-iris model compared to at the silver-iris model was affected by a focal fish's body size and the amount of time it spent expressing black-iris itself. Size ranges of live fish compared to model guppies for the three panels are i) 3.5-10 mm smaller; ii) ±3.5 mm iii) 3.5-11 mm larger. Lines and panels are based on predictions extracted from the two-way interaction term in a binomial GLMM. iv) Example pair of colour-calibrated model guppies used in each of the robotic experiment trials: silver-iris (above) and black-iris (below) versions of the same pair are shown. There were no effects of population on either the signaller or the receiver behaviours reported above.

Supplemental information: Dynamic eye colour as an honest signal of aggression

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Table S1. Summary information from the four populations used in this study. Mean female size calculated from the 2016 field season (see text).

Population	Resource availability	GPS coordinates	Mean female size ± standard deviation (mm)
Guanapo (Lower)	High	10°39.50’ N 61°15.20’ W	19.5±3.4
Guanapo (Upper)	Low	10°41.60’ N 61°15.80’ W	21.9±4.4
Aripo (Lower)	High	10°39.04’ N 61°13.38’ W	20.3±3.5
Aripo (Upper)	Low	10°41.74’ N 61°12.41’ W	21.6±5.0

Study species

Trinidadian guppies have been a model system in evolutionary biology since they inhabit multiple river basins across the island of Trinidad that differ repeatedly in resource distribution and predation pressure [S1]. Specifically, waterfalls act as selective barriers to the guppies’ major predators; piscivorous fish such as the pike cichlid (*Crenicichla frenata*), two-spot sardine (*Astyanax bimaculatus*), and wolf fish (*Hoplias malabaricus*) [S1]. The reduced predation pressure above these barrier waterfalls has led to populations reaching much higher densities, which combined with the lower primary productivity, leads to population size being largely limited by intra-specific competition for food [S2, S3]. Consequently, guppies provide an ideal system for studying the function and evolution of a putative signal used in intra-specific competition for food.

Staged behavioural interaction experiment

Fish capture and maintenance

Between February 2nd and April 14th 2016 we collected 288 guppies (144 of each sex) using seine nets from two populations (a low and high-resource regime [S2]) within each of the Aripo and Guanapo rivers in the Northern Range mountains on Trinidad (see Table S1).

Captured fish were transferred the same day to indoor aquaria at the St Augustine campus of the University of the West Indies, Trinidad and Tobago. Fish from each population were then split into same-sex trios. Guppies frequently form sex-assortative associations in the wild [S4] and feeding aggression by fish is largely between same-sex individuals (R.J.P.H. *personal observation*). To allow rapid individual identification of each fish during a trial, female trios were composed of visibly-different sized individuals chosen from the sexually-mature females caught from that population. Because of their lower variation in adult body size, individuals within male trios were identified based on their unique lateral colouration, which varies considerably between male guppies [S5]. Each trio was then placed in separate aquaria measuring 19 × 21 cm, with 10 cm deep water, and provided with a single refuge (2 rocks ~6 cm diameter stacked against one another) at one end of their aquarium, and allowed to settle for three days whilst being fed *ad-libitum* with fish flakes (Tetramin Tropical Fish flakes, Tetra™). Water temperature in all aquaria was maintained at 24°C with a 12:12hr day:night cycle. After acclimatising for three days, feeding was stopped for 24 hours prior to trials commencing to ensure fish were adequately motivated to feed (*sensu* [S6]).

Trials

To encourage simultaneous feeding, a single ‘food ball’, made from coating a circular stone (~2 cm diameter) with a gelatine and fish flake mix (Tetramin Tropical fish flakes, Tetra™), was lowered into the aquarium on the opposite side to the refuge using transparent monofilament fishing line. Trials lasted five minutes, and data collection began when at least two fish started feeding, after which continuous observations were made on the iris colour and agonistic behaviours of each fish. The agonistic behaviours recorded were ‘nips’, ‘nudges’, ‘tail beats’, ‘chases’ and ‘retreats’ (see [S6] for ethogram of these behaviours), with both the initiator and receiver being recorded for each. In all observed agonistic interactions, the individual that performed the aggressive behaviour caused the rapid retreat of the receiver of the behaviour. Data for all trials were collected by four experienced observers (R.J.P.H., A.M.B., P.R.L., L.C.N.-J.), blind to the population of origin of each trio. We ran a total of 12 trials for each sex within each population (i.e. 96 trials with 288 fish in total). After trials were completed, fish were anaesthetised in a solution of MS222 (Sigma Aldrich) and photographed against a 2 cm ruler to allow calculation of their standard body length (measured from the tip of the nose to the most posterior part of the caudal muscle, excluding the caudal fin) using ImageJ. After being photographed, we allowed fish to recover from their anaesthesia before releasing them into a pond within the St. Augustine campus at the University of the West Indies.

Robotic guppy food patch monopolisation experiment

After determining the behavioural correlates of eye colour in our staged trials, we developed a novel robotic system to disentangle whether eye colour itself is important in influencing the behaviour of signal receivers. Our robotic system used a combination of visually-realistic model guppies with an automated motorised system to mimic the monopolisation behaviour exhibited by guppies when defending a valuable resource such as a food patch. ‘Monopolisation’ is characterised by a single fish maintaining its position directly above or adjacent to a food patch, aggressively defending it from any approaching conspecifics [S6]. In previous studies this behaviour was often (but not exclusively) characterised by fish expressing black-irises [S6].

To create the model guppies for the robotic experiment, we first made three-dimensional model fish using polyurethane ‘fast-cast’ resin casts from RTV silicone moulds (Maragon, Hatfield, UK). The silicone moulds for these casts were made from 40 euthanised female fish caught in February 2017 (10 from each of our study populations), that were within 2 mm of the mean body length of adult females from each population (mean body size of each population determined from fish caught during the 2016 field season, details included in Table S1). The fish were euthanised using MS222 (Sigma Aldrich) and death was confirmed with pithing.

Calibration of model fish colouration

To make the guppy casts visually-realistic, we printed calibrated images from photographs using a visual model and adhered these to the casts. Using this novel technique, it is possible to make visually-calibrated models with the colouration and patterns that match the species-specific spectral sensitivities of any animal that has had its visual system modelled. Guppies exhibit large degrees of inter-population variation in their vision due to the plasticity in opsin-gene expression driven by differing lighting environments experienced during ontogeny [S7]. As our study involved multiple guppy populations, we therefore used the visual-model of our calibrated camera, which displays broad spectral peaks (see [S8]), allowing us to accommodate for this inter-population variation.

We photographed live fish side-on using a colour-calibrated camera (Samsung NX1000 with Nikkor EL 80 mm lens; the camera was converted to full spectrum and a Baader UV/IR cut filter was used to photograph in the human-visible range). Fish used for the photographs were lab-bred descendants of individuals collected in the lower part of the Aripo River (high predation/high productivity zone) in Trinidad (10°39'031'N; 61°13'40'W). We studied female fish in this particular experiment as they do not produce UV-reflecting ornaments (R.J.P.H. & J.T. *personal observations*), which cannot be printed at present. In future studies that include male guppies or other species that reflect UV, reflective dyes or paints could be manually applied to UV-ornamented areas (e.g. [S9]) on calibrated models.

Two grey standards made from sintered PTFE (Zenith 5% and 95%) were included in each photograph to control for lighting conditions and convert the image to linear, normalised reflectance [S8]. Our camera and printer (a Hewlett Packard M551 laser printer fitted with standard HP 507 ink cartridges) were able to produce calibrated colours in the human-visible range (400-700 nm, see [S8] for spectral sensitivity curves). Our system was colour-calibrated by printing a calibration sheet onto the same type of PVC film used to colour the fish models. The calibration sheet contained 1,026 squares, each having a colour coded by a randomly generated RGB value. The printed film was placed onto a flat surface spray-painted with White Plastic Paint (PlastiKote, Wolvega). The calibration sheet was photographed using the same calibrated camera and grey reflectance standard used for photographing the fish. Next, the normalised, linear pixel values measured by the camera for each of the 1,026 squares were calculated using the micaToolbox for ImageJ [S8, S10]. The RGB value of each square was converted from sRGB space (8 bits per channel) to linear RGB values. Finally, a polynomial model was fitted for each camera channel between the camera-measured and known linear RGB values. These polynomial models are similar to the methods used to convert between calibrated camera images and animal cone-catch quanta (see [S8]). Once the polynomial models were generated, these were used to create colour-calibrated linear RGB images (in 32-bits per channel) from the calibrated fish photographs (also 32-bits per channel). These linear RGB values were then converted back to sRGB-space (8 bits per channel). The image calibration processing was all performed using custom-written code in ImageJ (available on request). While this colour calibration process recreates colours faithfully for standard opaque objects, we found that the translucent bodies of real guppies resulted in their being brighter underwater than the opaque calibrated printouts. We therefore increased the brightness of the images until they matched that of live guppies that had been photographed underwater (all images were increased in brightness by the same level, ensuring there were no differences between treatments).

To create black- and silver-iris models, photographs were taken of each live guppy when they expressed silver-irises and subsequently when they expressed black-irises following induction of anaesthesia with MS222, which causes the body melanophores (including those in the iris) to dilate. Because black-iris expressed during aggressive interactions is not associated with corresponding body melanophore expression (R.J.P.H. *personal observation*) as they are when treated with MS222, we created our corresponding silver-iris and black-iris model treatments by using GIMP image software to 'cut' out the black irises from the MS222 treated fish photograph, and superimposing these onto a photograph of the same fish that was expressing normal body pigmentation (i.e. had not been treated with MS222). To control for any effects that the photographic manipulation procedure may have had, we created the silver-iris models using exactly the same procedure, but superimposing the eye from a different photograph of the same fish when it was still expressing silver-irises. Following their photographs, guppies were immediately euthanised with an overdose of MS222, with death confirmed by pithing, whilst still under anaesthesia.

Finally, the colour-calibrated guppy images were size-matched to the body size of the polyurethane cast guppies, and then printed onto the PVC film. We then spray-painted the resin casts with the same spray paint used to calibrate the models (White Plastic Paint (PlastiKote, Wolvega)), ensuring the correct background colour was used for calibration and model creation. After being sprayed, we wrapped the calibrated PVC film around the fish casts (with the printed-side against the model), ensuring that the lateral images deformed flexibly around the fish to faithfully match the fish colouration and patterning to its appropriate anatomy. The wet spray paint meant that the PVC film strongly adhered to the models once it had set, forming a waterproof ‘skin’. Before trials commenced, all models were submerged in aged (de-chlorinated) tap water for 12 hours before experiments started. Each silicone mould was used to make an otherwise-identical black- and silver-iris version of the same fish; we therefore made 10 black-iris and 10 silver-iris models for each fish population (80 in total).

Robotic validation experiment

Guppies are extremely social and strongly attracted to conspecifics. We exploited this behaviour to validate that the models were being perceived as conspecifics by running two binary shoal-choice experiments consisting of artificial shoals composed of two robotic guppies. In the first experiment, the preference for a shoal of visually-calibrated models was compared to a stepper motor rig without any guppy models, and the second experiment compared a shoal of visually-calibrated models to a shoal of two guppy models without the colour-calibrated PVC film attached (i.e. they were white). The first experiment allowed us to determine shoaling attraction to the artificial shoals, and the second experiment confirmed that this attraction was due to the colouration of the models as opposed to their shape and movement.

The artificial shoals were made by attaching two models 30 mm apart to a 450 mm long transparent loop of 7 lb monofilament fishing line, which was then wrapped around two 35 mm diameter pulleys. Both the pulleys were then submerged and attached to one side of the experimental tank (see below). The artificial shoal was then made to move in a standardised manner by subsequently attaching one of the pulleys to a dorsally placed stepper motor (via another loop of monofilament fishing line). The stepper motor was set to run on a continuous sequence 10ms between each step on a looped sequence, ensuring the shoal moved continuously with a smooth motion.

The aquaria used for the binary choice arena measured 400 × 300 × 200 mm, filled to 150 mm with aged (dechlorinated) tap water. We attached opaque plastic sheeting to the inside of the aquarium to avoid perimeter-pacing by fishing attempting to swim through the glass. At one end of the aquaria we attached the shoal of robo-guppies, and at the other we attached the same rig but without any robo-guppies (i.e. a monofilament loop of line, without any robo-guppies, attached to a dorsally-mounted stepper motor that was set to run with the same settings as the guppy shoal). We divided the arena into three separate zones: a central ‘neutral zone’ and two ‘preference zones’, the latter being 100 mm wide (i.e. less than four body lengths of the adult female guppies used in this experiment, which is their preferred shoaling distance to conspecifics [S11]). In the middle of the neutral zone we placed an opaque, cylindrical refuge (60 mm diameter) made from black acetate plastic, that could be remotely lifted and lowered due to being attached to a dorsally-mounted pulley via fishing line. Trials were filmed using a dorsally-mounted video-camera, allowing fish position at any time to be scored later from video analysis.

Validation trials

We used a total of 44 sexually mature female guppies caught from the Upper Guanapo River in Trinidad in March 2017 for the validation trials (24 females used in the visually-calibrated shoal vs empty tank control experiment, and 20 females in the calibrated vs resin-only model control experiment; GPS coordinates for population in Table S1). Each fish was used once for each trial. Each trial consisted of a single female guppy, which was introduced into the binary choice arena by netting and being placed into the acetate refuge and then allowed to settle for three minutes. Just prior to being introduced to the aquaria, the stepper motors that controlled the robo-guppy shoal and the control side were switched on. After a three-minute habituation time, the acetate cylinder was gently lifted 30 mm off the bottom of the tank, allowing the guppy to exit, after which its location was then recorded continuously for a total of five minutes. To control for any possible side biases in the test tank, we alternated the side that had the robo-guppy shoal vs the control after each trial. Water changes were performed after every second trial. The amount of time that the fish spent in each preference zone (in seconds) was then analysed using a paired t-test in R statistical software after checking the data conformed to parametric assumptions. One fish from the visually-calibrated shoal vs empty tank experiment was excluded from the analysis since video assessment showed it immediately sheltered in the acetate refuge after briefly emerging, and thus never entered either of the critical zones.

Fish showed a strong preference for associating with a shoal of robotic fish compared to the blank control (67.1% +/- 3.5 (mean percentage +/- standard error preference for the robo-guppy shoal); paired t-test: $t = 4.65$, $df = 22$, $p < 0.001$), and also for the shoal of visually calibrated models vs the resin-only models (64.8% +/- 5.3 (mean percentage +/- standard error preference for the calibrated robo-guppy shoal); paired t-test: $t = 2.91$, $df = 19$, $p < 0.009$).

Feeding competition based on iris-colour experiment

After confirming that female guppies strongly associate with the robotic fish, we created a rig designed to mimic the monopolisation behaviour described above and in the main text. We attached two robo-guppies to either end of a 35 cm long wooden dowel (10 mm diameter). Robo-guppies were each attached with superglue (Krazy Glue®) to the dowel with monofilament fishing line at their head and the base of their tail so that each model was 10 cm apart (which constitutes a greater distance than monopolising fish are observed to initiate attacks from; R.J.P.H. *personal observation*) and facing one another. The centre of the dowel was then attached directly to the shaft of a 4-phase 1.8 degree stepper-motor (PC Control Ltd, Kettering). The stepper motor was programmed to move clockwise and then anticlockwise 6 steps each (equivalent to spinning approximately 10°), three times and then followed by a three second pause before the sequence was repeated. The ultimate effect made the robo-guppies move in a similar manner to that of a monopolising fish that maintains a stationary position with intermittent undulations of the body directly above a food resource (see Figure S1). We made a total of 10 unique rigs for each population, each with the corresponding black and silver-iris model made from the same single sacrificed female guppy.

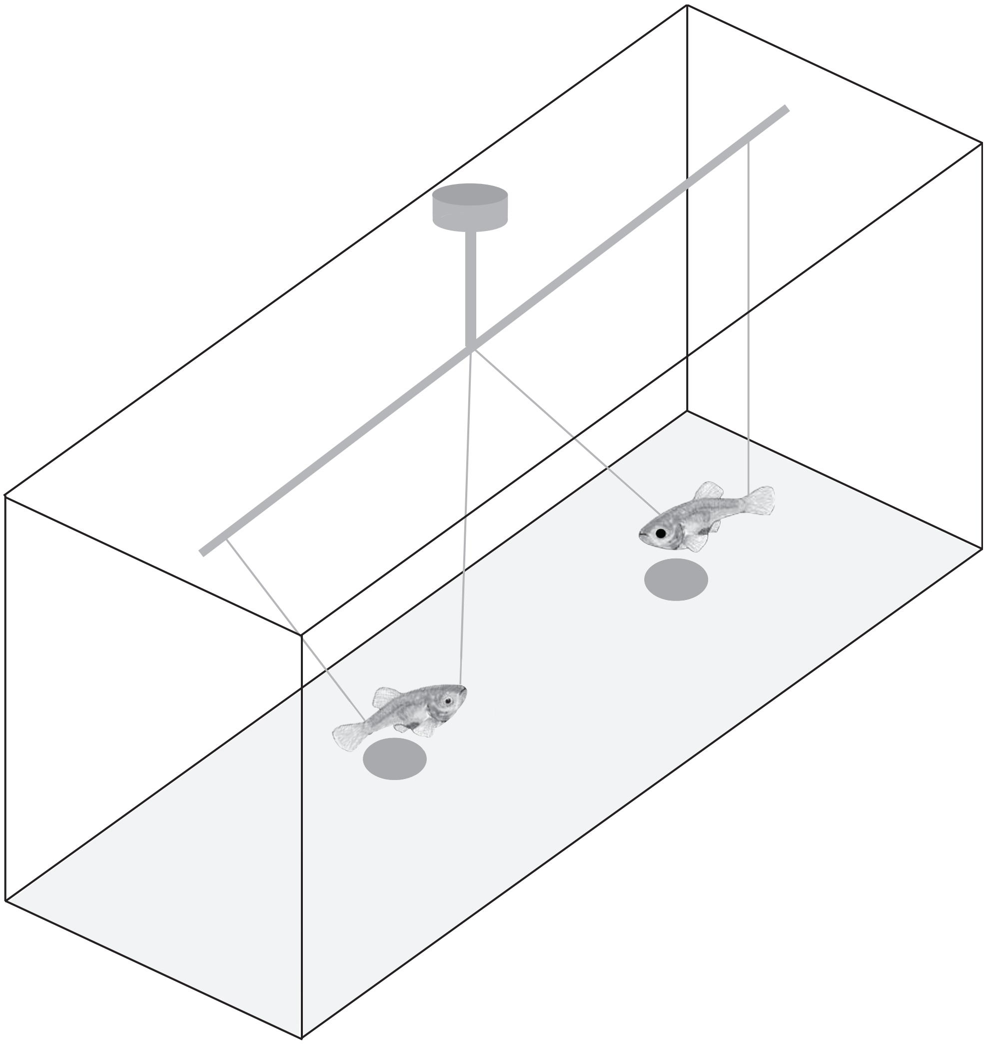

Figure S1. Binary choice arena showing the robotic guppy setup. Each rig had both a black and silver-iris model attached to opposite ends of a 350 mm long wooden dowel, which was then attached to a stepper motor that rotated briefly back and forth to simulate the swimming behaviour shown by monopolising guppies that are guarding a food patch (grey ovals underneath the guppy drawings in the diagram).

Between February 21st and May 21st 2017 we collected ~250 guppies of both sexes from each of the same locations and in the same manner as the fish collected for the staged behavioural observations (Table 1). Fish from each population were maintained in separate mixed-sex tanks (150 × 40 × 40 cm).

We ran a total of 20 trials for each population (i.e. 80 trials with 160 fish in total). In each trial we used two adult female guppies that differed in size by at least 1 mm (a large enough difference to allow rapid visual discrimination during trials). All trials were carried out in an aquarium (450 × 230 × 100 mm; Figure 1). Before a trial, two food balls were made by covering two identical sized 30 × 20 mm cylindrical lead fishing weights in a gelatine-fish flake mix (Tetramin Tropical fish-flakes). A food ball was then placed on the floor of the test tank directly underneath each robo-guppy, and the entire rig with the robo-guppies attached was lowered to 30 mm above the top of these to mimic the monopolisation behaviour described earlier.

For each trial, both test fish were netted from their home tank and placed into a circular refuge made from 6 cm diameter opaque black plastic acetate with a single large stone (~5 cm diameter) at the bottom and allowed to habituate for three minutes. After this period, the plastic barrier was remotely lifted slowly (using monofilament line attached to an overhead pulley) until it was 30 mm off the aquarium floor to allow the fish to exit the refuge. If fish showed a freeze or startle in response to the refuge moving then the trial was terminated and restarted with different fish. The robo-guppy stepper motor programme was started just prior to fish being placed into the aquarium. A trial was started as soon as at least one fish moved to within 5 cm of one of the food patches, and during trials the iris colour (black or silver) of each fish was recorded continuously along with the amount of time they spent feeding at either of the food patches. If fish did not feed at a food patch after 15 minutes then the trial was aborted and restarted with a different pair of fish. After their trial, fish were removed from the test tank and digitally photographed next to a 50 mm ruler to allow their standard body length to be measured using ImageJ as above.

Statistical analysis

Staged behavioural interaction experiment

To determine what predicts the likelihood of performing an aggressive behaviour, we created a binary outcome (aggressor=1; retreater=0) from each agonistic interaction to create the response variable in binomial generalised linear (mixed) models (one for each sex: due to issues with model convergence sex could not be included as a fixed effect). In both models we used the following predictors: eye colour (silver or black), the competitive origin (high or low), difference in standard body length between agonists, the number of previous wins and losses experienced by that individual, and an interaction between competitive origin, body size and eye colour. We controlled for differences between river basins by nesting competitive environment within river and including this as a random effect. The unique fish ID as well as the unique interaction ID were also included as random effects to control for pseudo-replication due to repeated observations of the same individual and pair. We tested for inter-observer differences in scoring the different behaviours by including the observer ID as a fixed effect in all models. After finding no significant differences between the observers, we re-ran our models after removing observer ID (results for the minimal models included in the main text).

Robotic guppy food patch monopolisation experiment

In our receiver response experiment, we used the number of seconds that each fish spent feeding at different food patches adjacent to either the black- or silver-iris robots as our binomial response variable. In this model, our predictors were difference in standard body length between the fish and the models, the proportion of time during the entire trial a fish spent expressing black-iris, the competitive environment the fish originated from, and the interaction between these three variables. We also included river, the unique robo-guppy rig ID (since each rig was used twice), and each trial's ID as random effects. Due to overdispersion, we also included an observation-level random effect to model the scale parameter [S12]. Results for this model are in the main text. Whilst we never observed any monopolisation from the focal fish at the food patches, we also ran two separate intercept-only binomial GLMM models to confirm that the treatment-related effects we observed were not being partially driven by one of the test fish avoiding the site being fed at by its test partner. In one model, the binary response variable was whether the large or small fish fed first (1=large, 0=small) to determine any priority effects that might imply dominance behaviour. In the second model, the binary response variable was whether the second fish to feed fed at either the same (=1) or different (=0) food patch as its partner. In both models we included the unique robotic rig, population of origin, and also the unique fish ID (to control for multiple observations per fish) as random effects. There was no significant effect of priority in which fish fed in all our trials (Z score: -0.23, $p = 0.818$), and in our second model the second fish to feed was significantly more likely to feed at same foraging patch as its social partner (Z score: 5.36, $p < 0.001$).

All GLMMs were analysed using the lme4 package [S13] in R version 3.3.3 statistical software [S14]. We obtained our optimal models by sequentially dropping non-significant terms, with each term's significance determined using likelihood ratio tests to compare nested models with and without the term of interest.

Ethical note

The behavioural aggression trials run in this study were approved by the Psychology Ethical Review Group at the University of Exeter, and the photography involved in the robotic development carried out under a UK Home Office Project License (number 30/3308).

Data availability

The data used in this study are available upon request.

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Author Contributions

R.J.P.H., D.P.C. and S.K.D. designed the study. R.J.P.H. and J.T. developed the visually-calibrated guppy models in discussion with D.P.C. R.J.P.H. coordinated fieldwork and experiments in Trinidad. R.J.P.H., M.R.M.L., A.M.B., P.L., L.C.N.-J. and H.E.A.M. collected the data. I.R. contributed materials and provided infrastructure whilst in Trinidad. R.J.P.H. analysed the data, in discussion with D.P.C. and S.K.D. R.J.P.H. wrote the first draft of the manuscript, R.J.P.H., D.P.C. and SKD wrote subsequent drafts, and all authors contributed to the final version of the manuscript.

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