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Dynamic eye colour as an honest signal of aggression

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Animal eyes are some of the most widely recognisable structures in nature. Due to their salience to predators and prey, most research has focused 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. Furthermore, using a novel, visually realistic robotic system to create a mismatch between signal and relative competitive ability, we show that eye colour is used to honestly signal aggressive motivation. Specifically, robotic 'cheats' (that is, smaller, less-competitive robotic fish that display 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 biomimetic robotic system for investigating animal behaviour, and provides experimental evidence that socially mediated costs towards low-quality individuals may maintain the honesty of dynamic colour signals.

Many species are extremely attuned to eyes and eye-like stimuli, particularly in predatory contexts [1], and this sensitivity may predispose eyes and their

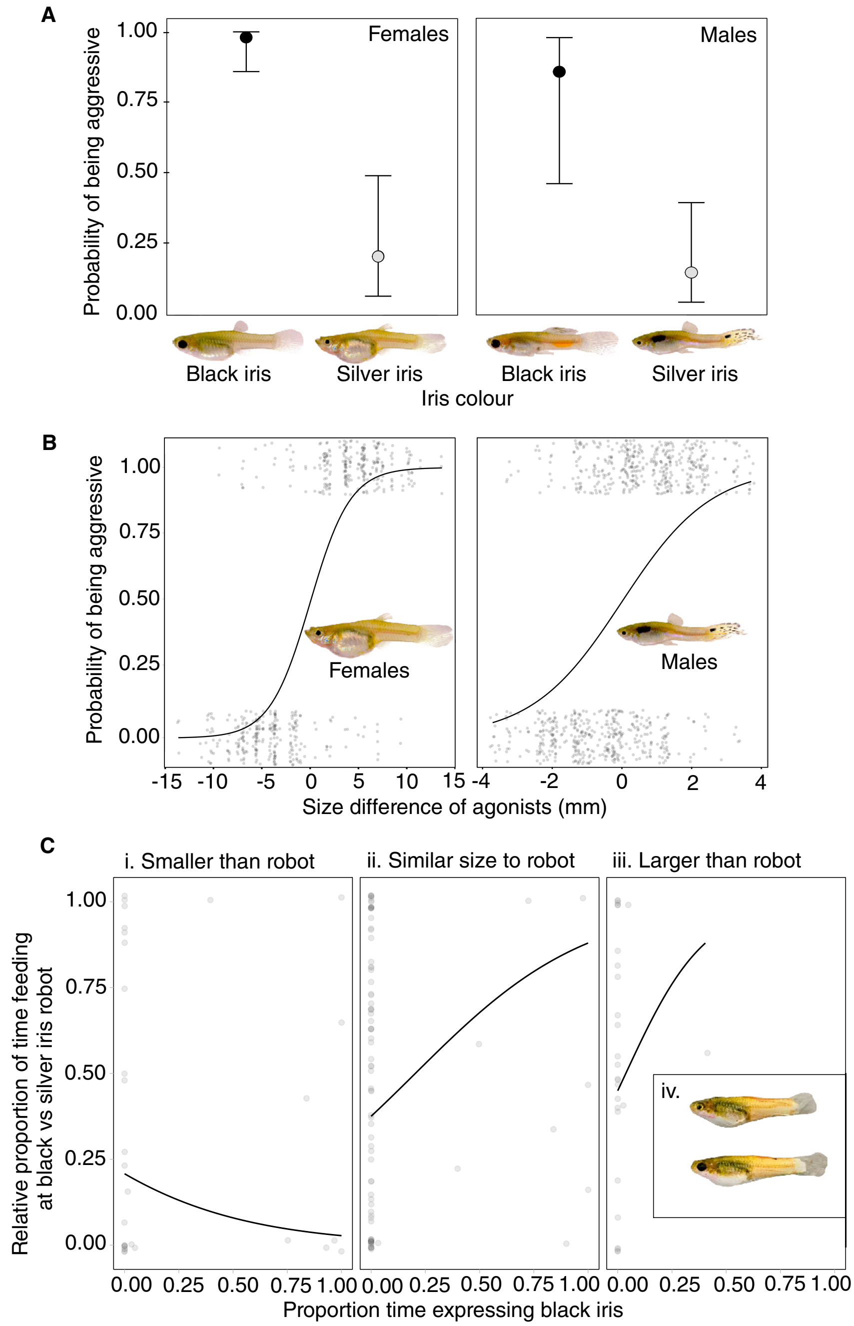

Figure 1. Effect of iris colour and body size on signaller and receiver behaviours.
Iris colour (A) and body size (B) predict the probability of performing (versus receiving) an aggressive behaviour during agonistic interactions in guppies. Circles and error bars denote mean and standard error within each treatment. 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. Regression lines in panels B and C are based on predictions extracted from binomial GLMMs. 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.

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, increasing the conspicuousness of their eyes, which 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]. Although theoretical studies on the evolution and maintenance of dynamic signals suggest that 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 (see details in Table S1 of the Supplemental Information). 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, χ^2=51.36, p<0.001; males, OR=25.8, χ^2=20.82, p<0.001; Figure 1A. Body size in females, OR=2.7, χ^2=105.77, p<0.001; males, OR=3.4, χ^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 utilises a novel method of creating three-dimensional model guppies with visually realistic colouration and patterns based on calibrated photographs (Figure 1C; see Supplemental Experimental Procedures 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 the 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 (that is, 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, χ^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 the costs of 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.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and one table, experimental procedures, acknowledgments and author contributions, and can be found with this article online at https://doi.org/10.1016/j.cub.2018.04.078.

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