Social selection maintains honesty of a dynamic visual signal in cichlid fish

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INTRODUCTION

Our understanding of animal communication has been driven by advances in theory, not least because empirical evidence has been difficult to obtain [1]. Costly signaling theory is the dominant paradigm explaining the evolution of honest communication [2–5]. According to it, honesty is maintained by imposing different strategic costs on signals produced by animals of different qualities (e.g. handicaps and indices) [6–8]. In spite of generalized acceptance, other models have suggested that strategic costs at equilibrium are alone not sufficient, nor even necessary, for reliable signaling [9–11]. One alternative solution to the puzzling evolution of honest signals comes from potential, rather than realized costs, imposed by receivers. Indeed several theoretical models suggest that honesty of signaling systems can instead be socially-enforced and context-dependent. In this case animals that signal reliably do not need to incur any strategic costs on top of the efficacy costs that signal transmission entails [12–14]. These
conclusions require that empiricists measure marginal costs of cheating in manipulated out-of-equilibrium signals where individuals are forced to exhibit unreliable signal expression [13, 15–18]. Here we quantify the signaling efficacy and message of the facial color pattern in the Princess of Burundi cichlid (*Neolamprologus brichardi*) using theoretical visual models and staged dyadic combats. By manipulating signal expression and simulating a cheater invasion we demonstrate that social selection promotes the honesty of this dynamic conventional signal with low production costs. By directly probing the sender of a signal, social selection is likely to be the mechanism of choice shaping the evolution of cheap context-dependent signals. In the same way that sexual selection drives the evolution of coloration in dichromatic species [19], we suggest that social selection can affect the evolution of pigmentation patterns in sexually monochromatic cichlid species, with potential impacts on diversification dynamics.

RESULTS AND DISCUSSION

**A new framework for studying intraspecific color signals.** The understanding of honest signaling in animal communication centers on the costs of expressing a signal, yet it remains unclear whether signaling costs have even been determined empirically (e.g. [17, 18, 20]). Here we combine conceptual approaches from visual modeling and signaling theory (e.g. [21]) into a new 3-stage framework that generates objective predictions about the evolution of reliable color signals, making the demonstration of the existence of strategic costs a more tractable empirical problem (Figure 1). We follow Higham’s [17] definition of costly signaling, where cost functions can be zero at the equilibrium, to include social selection through punishment as a mechanism that can generate marginal costs to cheaters and maintain signaling reliability (as elaborated elsewhere [20]). Our approach (Figure 1), which can be readily extended to other
species, first requires using visual models to formally quantify signal efficacy and identify the
correct target of communication. We then use behavior observations and assays to determine the
message conveyed by our signal of interest. Finally, we identify which class of costs unreliable
signaling might incur, by experimentally manipulating sender signals out-of-equilibrium and
recording receiver’s reactions. Here we follow Fraser’s [20] classification based on intrinsic and
imposed costs to determine whether liar detection mechanisms exist. Liar detection is expected
to evolve in cheap conventional signals where receivers can immediately probe senders, but not
in intrinsically costly handicaps or indices in which case reliability is verified far into the future
in terms of viability and fecundity [13].

Using this framework we explored the evolution of the facial color mask in the cichlid
fish *N. brichardi* (Figure 2). We chose this system because it is a lifelong territorial species with
elaborate social habits for which considerable behavioral and ecological information is available
(see Supplemental Experimental Procedures). It is a sexually monochromatic (i.e. both sexes
look alike) substrate brooder of the species-rich tribe Lamprologini from East African Lake
Tanganyika [22] and has emerged as a model in cooperative breeding studies [23]. The dominant
couple has the peculiarity of being aided by several subordinate helpers in these tasks, organized
in a linear hierarchy. Their rocky territory is a valuable resource that simultaneously provides
substrate for reproduction and shelter against predation. Hence, losing access to a shelter has a
strong negative impact on fitness and survival. As a consequence of cooperative breeding and
colony life, individuals repeatedly and regularly interact, which creates optimal conditions for
the evolution of context-dependent signaling by individuals of both sexes and different ages
throughout their lives.
**Stage 1: High chromatic conspicuousness of *N. brichardi’s* facial coloration.** Unambiguous communication selects for signaling systems that promote effective stimulation of sensory systems relative to environmental noise and signal degradation. Such high conspicuousness to intended receivers is achieved by stimulation of adjacent photoreceptors in opposite ways by complementary radiance spectra [24–26]. Design strategies for increased conspicuousness and transmission efficacy thus include the use of (i) white or highly reflective colors adjacent to dark patches, (ii) adjacent patches with complementary colors and (iii) color combinations centered or just offset transmission maximum of the medium [24, 26, 27]. Further, a visual signal in a particular light environment is most conspicuous when adjacent color elements have greater contrasts than non-adjacent elements [27–30].

Using spectral reflectance measurements and theoretical fish visual models, we show that the facial color pattern in aggressive, dominant *N. brichardi* achieves high chromatic conspicuousness to the visual system of conspecifics by following all three predictions (Figure 3A and 3C, filled circles; Figure S1). This signal design is exceptionally effective and ensures transmission efficacy in the aquatic environment: white is a broadband optical reflector, reflecting across all the available light spectrum and structural blue patches reflect the high-intensity wavelengths available underwater, while the adjacent black melanic stripes absorb most incident light. Chromatic contrast is further achieved by use of complementary colors, blue and yellow, centered in the highest light intensity of water transmission. As such, chromatic contrasts differ significantly between adjacent and non-adjacent patches (linear mixed-effects model [LMM]: $F_{1,9} = 207.31, P < 0.001$) and all pairwise color comparisons are well above the just noticeable difference (JND) threshold of one, a standard in chromatic color discrimination [29, 31]; Figure 3A and 3C, filled circles). Compared to chromatic contrasts, achromatic contrasts do
not seem to contribute to pattern conspicuousness, as adjacent and non-adjacent elements do not significantly differ in luminance from one another (LMM: $F_{1,9} = 4.61, P = 0.06$; Figure 3D, filled circles).

Stage 2: *Neolamprologus brichardi* make context-dependent use of facial signal. High chromatic conspicuousness of facial patterns implicates selection for unambiguous signaling, at least at close range (Figure 1). We thus tested its function in communication by staging dyadic combats of territory-holding fish. As expected, body size (LMM: $F_{1,18} = 8.02, P = 0.01$) and fighting ability (LMM: $F_{1,18} = 67.31, P < 0.001$) determine the outcome of staged combats, irrespective of sex (LMM: $F_{1,18} = 1.85, P = 0.19$ and LMM: $F_{1,18} = 0.04, P = 0.85$; Figure S2A and S2B). Most importantly, we found that a change in aggressive intent by losers of the combat leads to a rapid paling of the horizontal facial stripe at the end of the contest (generalized linear mixed-effects model [GLMM] with binomial error distribution: $\chi^2 = 14.97, P < 0.001$; Figures 3B, 4A, S1G). Hence stripe intensity at the end of the combat reflects motivation to fight and aggressive intent, while stripe darkness at the beginning does not influence contest outcome (GLMM with binomial error distribution: $\chi^2 = 0.01, P = 0.93$), which is fundamentally different from other well-described signals that function as badges of status [31]. Therefore, rapid paling of the horizontal facial stripe may be used to instantaneously signal an individual’s intent to fight and dominance. Such rapid movement of pigments within melanophores (black pigment cells) is a physiological response available to many lower vertebrates (e.g. fish, reptiles) and invertebrates (e.g. cephalopods), and can occur within a few seconds in fish [32, 33].

Next, we used theoretical visual models to test whether the physiological paling of the horizontal stripe induces changes in conspicuousness of the overall facial pattern. We found that
even after paling takes place chromatic conspicuousness is unaffected (empty circles in Figures 3C and S1H). In particular, high chromatic contrast is achieved by adjacent and non-adjacent signal design (LMM: $F_{1,18} = 208.21, P < 0.001$) and not by stripe darkness (LMM: $F_{1,18} = 3.48, P = 0.08$) or interaction between the two fixed effects (LMM: $F_{1,18} = 0.05, P = 0.82$). This model explains 99.31% of chromatic contrast variance, 96.50% of which is explained by adjacency of the color elements, while changes in horizontal stripe luminance explain the remaining variance. On the other hand, we found that achromatic contrasts are strongly influenced by changes in luminance of the horizontal stripe (LMM: $F_{1,18} = 9.11, P = 0.007$), as the balance between adjacent and non-adjacent contrasts (LMM: $F_{1,18} = 5.07, P = 0.037$) and the interaction between the two becomes important (LMM: $F_{1,18} = 6.78, P = 0.018$; empty circles in Figure 3D and S1I). This model explains 95.90% of the achromatic contrast variance, 68.53% of which is explained by changes in darkness of the horizontal stripe, 22.34% by signal design (patch adjacency) and the remainder 5.02% by their interaction. Thus, we find that white, yellow and blue are less dynamic elements of the facial color pattern, and seem to provide little or no information regarding changes in aggressive intent but instead act as amplifiers to enhance pattern conspicuousness.

Our visual models and behavioral experiments indicate that individuals use rapid physiological changes in luminance (achromatic contrast) of the horizontal stripe element to dynamically communicate reversals in aggressive intent and dominance, while the color pattern conspicuousness remains high at all times by virtue of its stable chromatic properties. Using this dual mechanism is an elegant way to ensure that conspicuousness, and hence communication efficacy, does not decrease due to context-dependent signaling. This constantly ‘on’ signaling strategy of aggressive intent in *N. brichardi* is unexpected as it is opposite to other signaling
systems such as in anoles lizards, chameleons, or transiently territorial fish which only briefly or seasonally display their signals during agonistic encounters [32, 34–36] (Figure 2). Our findings could possibly be explained by lifelong territoriality, constant interactions with conspecifics and different predation escape strategies of *N. brichardi*. While these stenotopic cichlids rely on their valuable rocky territories for shelter (and breeding) and conspicuously signal their ownership at all times, chameleons have to rely on immobility and camouflage to escape avian predation and become only momentarily conspicuous while displaying to conspecifics [35]. Instead, from a signaling perspective uninterrupted conspicuousness of *N. brichardi* is more similar to that of aposematic species, which rely on high conspicuousness to continuously signal their distastefulness [37, 38].

**Stage 3: Proximate mechanisms producing an evolutionary stable signaling strategy.** Using a dyadic combat experiment in combination with the visual models we showed that changes in luminance of the horizontal melanistic stripe are used during social agonistic interactions and correlate with aggressive intent. However, whether the fish directly respond to the physiological color changes of the horizontal facial stripe and if these changes are then used to assess another individual’s aggressive intentions needs direct behavioral evaluation. Moreover, if changes in luminance were to reliably signal aggressive intent, we would expect the existence of an honesty mechanism to minimize cheating opportunities. To test these expectations we simulated a cheater invasion of the signaling system, by manipulating luminance of the horizontal facial stripe out-of-equilibrium (via artificial darkening or paling; Figures S2C, S2D and Table S1) and presenting fish to their mirror images. Our setup is opposite to the commonly used approach of displaying manipulated individuals to focal territory owners as noted by Bradbury and
Vehrencamp \[3\], having the advantage of testing behavior of non-territorials (i.e. the receivers of the mirror image), which are the ones most interested in detecting unreliable signals if used by territorial, dominant individuals. As a null hypothesis (Figure 1), (1) we do not expect to observe differences in aggression toward manipulated or non-manipulated individuals if stripe intensity does not encode individual fighting abilities (but simply correlates with them). On the other hand, (2) if stripe intensity signals a contest-independent intrinsic quality whereby strategic costs guarantee honesty (e.g. handicap), subordinates should not challenge cheating individuals with enhanced signals but should do so toward individuals with subdued signals. Alternatively, (3) if stripe intensity signals contest-dependent dominance whereby social costs (i.e. punishment of cheaters) maintain signal honesty, we expect increased levels of aggression toward any unreliable signal (i.e. a conventional signal).

We found that receivers actively ‘read’ and react to manipulations of the horizontal facial stripe, recognizing and punishing cheaters (Figures 4B and S2E-G). Manipulation of the horizontal stripe had a significant effect on the number of aggressive bouts received (LMM: $F_{2,45} = 13.73$, $P < 0.001$), irrespective of sex (LMM: $F_{2,45} = 0.48$, $P = 0.62$). Individuals with darkened stripes received significantly more aggression than individuals with paled stripes (Tukey HSD: $z = -3.89$, $P < 0.001$) and controls (Tukey HSD: $z = -6.59$, $P < 0.001$). Importantly, individuals with paled stripes also received more aggression than controls (Tukey HSD: $z = -2.97$, $P = 0.008$), indicating that unreliable signaling brings increased marginal costs to all types of cheaters.

By manipulating the signal out-of-equilibrium we simultaneously show that physiological color changes are interpreted by receivers as a dynamic context-dependent signal of aggressive intent and that social selection by receiver retaliation is the proximate mechanism effectively
promoting the honesty of this visual signal (hypothesis 3, above). Thus, as with communication
efficacy, we demonstrate that communication reliability does not decrease due to context-
dependent signaling but is rather promoted by contest-dependent policing. Since aggressive
intent is not a quality that can be easily handicapped [3], receivers can directly assess reliability
of signals of aggressive intent with relative ease [13] and impose social costs on cheaters. Our
study provides rare empirical evidence that, similar to paper wasps [31, 39], fish are able to
detect and punish individuals who signal unreliably, be they cheaters signaling strength
(‘bluffers’) or modest liars (‘Trojans’). Interestingly, the fact that social selection against
cheaters is not symmetrical supports the view that signaling systems are more likely disrupted by
‘Trojans’ than by ‘bluffers’ [40]. We thus provide empirical support to theoretical models
concluding that honest communication does not require signals with differential strategic costs
and that reliability can indeed be guaranteed by mechanisms that promote low realized costs for
honest signalers, such as social selection [12–14, 16]. Hence, since receivers can effectively
probe reliability of signals in real time, we propose that social selection and cheap conventional
signals are expected to be a widely chosen solution for honest context-dependent signaling.

Physiological color changes have previously been implicated in signaling aggressive
intent in a number of taxa, in particular fish [32, 41–43]. Increased levels of aggression toward
the signal reported in some of these studies were interpreted as receiver retaliation costs
maintaining honesty of a conventional signal. We extend these findings and show that unreliable
signaling has increased costs relative to reliable signaling, which is pivotal to the evolution of
honest signals [2, 13, 17]. The rapid physiological color changes of this dynamic color signal
rival the morphological and behavioral context-dependent signaling strategies evolved by other
territorial species [44, 45] and allow the fish to instantaneously communicate their intention to
fight or retreat from the combat by reliably showing or concealing the signal of aggressive intent. Such dynamic expression noted among fish [46] contrasts with other more or less static visual signals, such as plumage badges of status in birds [47, 48], exoskeleton color patterns in insects [31], or aposematic signals in poison frogs [37], all of which are thought to signal more temporally stable characteristics of quality and distastefulness.

In summary, our framework for color signal analysis proved important in generating predictions according to trends emerging from theory. We demonstrated that the facial mask of *N. brichardi* has stable chromatic properties that keep signaling efficacy high at all times, while rapid physiological changes in luminance of just one element (the horizontal melanic stripe) communicate reversals in aggressive intent and dominance. We further demonstrated that social selection maintains honesty of the signaling system, which could be nature’s favored mechanism for promoting the honesty of dynamic visual signals with low production costs, such as those produced by physiological color changes. Together, these findings suggest that social selection may account for the dramatic diversity of color patterns (stripes, bars, blotches) we observe in many sexually monochromatic cichlid species endemic to Lake Tanganyika [22] and elsewhere, acting together with natural selection in shaping diversity in cichlid fishes [49, 50]. Social selection is expected to drive rapid signal evolution especially in isolated allopatric populations [14, 51], but until now research into color signaling in cichlids has centered on the sexually dichromatic assemblages from Lake Malawi and Lake Victoria [19]. Our results point to rapid social trait evolution as another process potentially affecting speciation dynamics in cichlids. Confirmation of its importance would bring social selection to the same level as sexual and natural selection in shaping adaptive radiations of cichlid fishes.
AUTHOR CONTRIBUTIONS

H.F.G. conceived the study and designed the experiments together with J.C.B., F.C. and W.S..

J.C.B., F.C. and H.F.G. performed the experiments and analyzed the data together with M.D.H.

All authors contributed to writing and discussion. All authors reviewed and approved the final version of the manuscript.

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**Figure 1. Proposed framework for studying color signals.** Flowchart of the conceptual framework proposed for studying intraspecific color signals generates predictions to determine signal efficacy, function and proximate reliability mechanisms.

**Figure 2. Dynamic animal visual signals.** Territorial species display a variety of conspicuous visual signals to communicate aggressive intent. To decrease predation pressure and in non-aggressive contexts several species use morphological, physiological or behavioral adaptations to conceal signals [26, 44, 45, 52]. We propose that rapid physiological color changes, achieved by pigment movement in melanophores (black pigment cells), are a cheap proximate mechanism turning a visual signal of aggressive intent ‘on’ or ‘off’ in lifelong territorial fish. Clockwise from top left: facial color pattern in dominant Princess of Burundi cichlid (*Neolamprologus brichardi*); extended dewlap in trunk-ground Brown Anole (*Anolis sagrei*); partially covered epaulette in Fan-tailed Widowbird (*Euplectes axillaris*)

**Figure 3. Color properties of facial elements in dominant and subordinate *Neolamprologus brichardi*.**
(A and B) Mean spectral reflectance of facial color patches. (A) Horizontal (green triangle) and vertical (black triangle) facial stripes have the same reflectance in dominant fish (note arrow). (B) Losing a combat and becoming subordinate significantly increases reflectance of horizontal facial stripe in subordinate fish, i.e. paling occurs. (See Figure S1G for 95% confidence intervals of spectral reflectance).

(C and D) Chromatic and achromatic contrasts (mean ± SEM) between pairs of adjacent and non-adjacent color patches as perceived by *N. brichardi*, ordered from highest to lowest in dominant fish. (C) High chromatic contrast $\Delta S$ is achieved by any combination of blue, yellow and black patches. (D) High achromatic contrast $\Delta L$ is achieved by combining black melanic stripes and other patches. Stippled line marks the 1 JND (just noticeable difference), threshold after which two colors are thought to be perceived as different [29, 53]. Asterisks illustrate significant differences in contrast between dominant and subordinate fish (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$). (Figure S1 shows data used to build visual models)

**Figure 4. Horizontal facial stripe provides reliable information on aggressive intent**

(A) Facial stripe intensity is associated with fighting ability (winning or losing) at end of combat. L: losers; W: winners.

(B) Social selection (aggressive bouts, mean ± SEM) on out-of-equilibrium signals and control. Unreliable signaling of strength (darkened stripe) and weakness (paled stripe) are punished by increased receiver retaliation costs relative to reliable signaling (control). Asterisks illustrate significant differences in facial stripe luminance at end of combat and of pairwise post-hoc tests between treatments (*** $P < 0.001$, ** $P < 0.01$). (See also Figure S2).
Characterization of trait properties relative to visual system of receiver

Determination of message encoded

Classification of signal based on reliability source

- color pattern
  - is color pattern conspicuous?
    - yes: communication
    - no: crypsis
  - context x
    - correlation with behaviors?
      - yes: experimental manipulation of trait out-of-equilibrium and test of receiver responses in same behavioral context
      - no: cheating is not detected
        - honest signals of quality without direct liar detection mechanisms
          - handicap, index
        - honest signals of state or motivation with direct liar detection
          - conventional signal
        - no function in communication