

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

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11

12 **INTRODUCTION**

13 Our understanding of animal communication has been driven by advances in theory, not least

14 because empirical evidence has been difficult to obtain [1]. Costly signaling theory is the

15 dominant paradigm explaining the evolution of honest communication [2–5]. According to it,

16 honesty is maintained by imposing different strategic costs on signals produced by animals of

17 different qualities (e.g. handicaps and indices) [6–8]. In spite of generalized acceptance, other

18 models have suggested that strategic costs at equilibrium are alone not sufficient, nor even

19 necessary, for reliable signaling [9–11]. One alternative solution to the puzzling evolution of

20 honest signals comes from potential, rather than realized costs, imposed by receivers. Indeed

21 several theoretical models suggest that honesty of signaling systems can instead be socially-

22 enforced and context-dependent. In this case animals that signal reliably do not need to incur any

23 strategic costs on top of the efficacy costs that signal transmission entails [12–14]. These

24 conclusions require that empiricists measure marginal costs of cheating in manipulated out-of-
25 equilibrium signals where individuals are forced to exhibit unreliable signal expression [13, 15–
26 18]. Here we quantify the signaling efficacy and message of the facial color pattern in the
27 Princess of Burundi cichlid (*Neolamprologus brichardi*) using theoretical visual models and
28 staged dyadic combats. By manipulating signal expression and simulating a cheater invasion we
29 demonstrate that social selection promotes the honesty of this dynamic conventional signal with
30 low production costs. By directly probing the sender of a signal, social selection is likely to be
31 the mechanism of choice shaping the evolution of cheap context-dependent signals. In the same
32 way that sexual selection drives the evolution of coloration in dichromatic species [19], we
33 suggest that social selection can affect the evolution of pigmentation patterns in sexually
34 monochromatic cichlid species, with potential impacts on diversification dynamics.

35

36 **RESULTS AND DISCUSSION**

37 **A new framework for studying intraspecific color signals.** The understanding of honest
38 signaling in animal communication centers on the costs of expressing a signal, yet it remains
39 unclear whether signaling costs have even been determined empirically (e.g. [17, 18, 20]). Here
40 we combine conceptual approaches from visual modeling and signaling theory (e.g. [21]) into a
41 new 3-stage framework that generates objective predictions about the evolution of reliable color
42 signals, making the demonstration of the existence of strategic costs a more tractable empirical
43 problem (Figure 1). We follow Higham's [17] definition of costly signaling, where cost
44 functions can be zero at the equilibrium, to include social selection through punishment as a
45 mechanism that can generate marginal costs to cheaters and maintain signaling reliability (as
46 elaborated elsewhere [20]). Our approach (Figure 1), which can be readily extended to other

47 species, first requires using visual models to formally quantify signal efficacy and identify the
48 correct target of communication. We then use behavior observations and assays to determine the
49 message conveyed by our signal of interest. Finally, we identify which class of costs unreliable
50 signaling might incur, by experimentally manipulating sender signals out-of-equilibrium and
51 recording receiver's reactions. Here we follow Fraser's [20] classification based on intrinsic and
52 imposed costs to determine whether liar detection mechanisms exist. Liar detection is expected
53 to evolve in cheap conventional signals where receivers can immediately probe senders, but not
54 in intrinsically costly handicaps or indices in which case reliability is verified far into the future
55 in terms of viability and fecundity [13].

56 Using this framework we explored the evolution of the facial color mask in the cichlid
57 fish *N. brichardi* (Figure 2). We chose this system because it is a lifelong territorial species with
58 elaborate social habits for which considerable behavioral and ecological information is available
59 (see Supplemental Experimental Procedures). It is a sexually monochromatic (i.e. both sexes
60 look alike) substrate brooder of the species-rich tribe Lamprologini from East African Lake
61 Tanganyika [22] and has emerged as a model in cooperative breeding studies [23]. The dominant
62 couple has the peculiarity of being aided by several subordinate helpers in these tasks, organized
63 in a linear hierarchy. Their rocky territory is a valuable resource that simultaneously provides
64 substrate for reproduction and shelter against predation. Hence, losing access to a shelter has a
65 strong negative impact on fitness and survival. As a consequence of cooperative breeding and
66 colony life, individuals repeatedly and regularly interact, which creates optimal conditions for
67 the evolution of context-dependent signaling by individuals of both sexes and different ages
68 throughout their lives.

69 **Stage 1: High chromatic conspicuousness of *N. brichardi*'s facial coloration.** Unambiguous
70 communication selects for signaling systems that promote effective stimulation of sensory
71 systems relative to environmental noise and signal degradation. Such high conspicuousness to
72 intended receivers is achieved by stimulation of adjacent photoreceptors in opposite ways by
73 complementary radiance spectra [24–26]. Design strategies for increased conspicuousness and
74 transmission efficacy thus include the use of (i) white or highly reflective colors adjacent to dark
75 patches, (ii) adjacent patches with complementary colors and (iii) color combinations centered or
76 just offset transmission maximum of the medium [24, 26, 27]. Further, a visual signal in a
77 particular light environment is most conspicuous when adjacent color elements have greater
78 contrasts than non-adjacent elements [27–30].

79 Using spectral reflectance measurements and theoretical fish visual models, we show that
80 the facial color pattern in aggressive, dominant *N. brichardi* achieves high chromatic
81 conspicuousness to the visual system of conspecifics by following all three predictions (Figure
82 3A and 3C, filled circles; Figure S1). This signal design is exceptionally effective and ensures
83 transmission efficacy in the aquatic environment: white is a broadband optical reflector,
84 reflecting across all the available light spectrum and structural blue patches reflect the high-
85 intensity wavelengths available underwater, while the adjacent black melanic stripes absorb most
86 incident light. Chromatic contrast is further achieved by use of complementary colors, blue and
87 yellow, centered in the highest light intensity of water transmission. As such, chromatic contrasts
88 differ significantly between adjacent and non-adjacent patches (linear mixed-effects model
89 [LMM]: $F_{1,9} = 207.31$, $P < 0.001$) and all pairwise color comparisons are well above the just
90 noticeable difference (JND) threshold of one, a standard in chromatic color discrimination [29,
91 31]; Figure 3A and 3C, filled circles). Compared to chromatic contrasts, achromatic contrasts do

92 not seem to contribute to pattern conspicuousness, as adjacent and non-adjacent elements do not
93 significantly differ in luminance from one another (LMM: $F_{1,9} = 4.61$, $P = 0.06$; Figure 3D, filled
94 circles).

95

96 **Stage 2: *Neolamprologus brichardi* make context-dependent use of facial signal.** High
97 chromatic conspicuousness of facial patterns implicates selection for unambiguous signaling, at
98 least at close range (Figure 1). We thus tested its function in communication by staging dyadic
99 combats of territory-holding fish. As expected, body size (LMM: $F_{1,18} = 8.02$, $P = 0.01$) and
100 fighting ability (LMM: $F_{1,18} = 67.31$, $P < 0.001$) determine the outcome of staged combats,
101 irrespective of sex (LMM: $F_{1,18} = 1.85$, $P = 0.19$ and LMM: $F_{1,18} = 0.04$, $P = 0.85$; Figure S2A
102 and S2B). Most importantly, we found that a change in aggressive intent by losers of the combat
103 leads to a rapid paling of the horizontal facial stripe at the end of the contest (generalized linear
104 mixed-effects model [GLMM] with binomial error distribution: $\chi^2_1 = 14.97$, $P < 0.001$; Figures
105 3B, 4A, S1G). Hence stripe intensity at the end of the combat reflects motivation to fight and
106 aggressive intent, while stripe darkness at the beginning does not influence contest outcome
107 (GLMM with binomial error distribution: $\chi^2_1 = 0.01$, $P = 0.93$), which is fundamentally different
108 from other well-described signals that function as badges of status [31]. Therefore, rapid paling
109 of the horizontal facial stripe may be used to instantaneously signal an individual's intent to fight
110 and dominance. Such rapid movement of pigments within melanophores (black pigment cells) is
111 a physiological response available to many lower vertebrates (e.g. fish, reptiles) and
112 invertebrates (e.g. cephalopods), and can occur within a few seconds in fish [32, 33].

113 Next, we used theoretical visual models to test whether the physiological paling of the
114 horizontal stripe induces changes in conspicuousness of the overall facial pattern. We found that

115 even after paling takes place chromatic conspicuousness is unaffected (empty circles in Figures
116 3C and S1H). In particular, high chromatic contrast is achieved by adjacent and non-adjacent
117 signal design (LMM: $F_{1,18} = 208.21$, $P < 0.001$) and not by stripe darkness (LMM: $F_{1,18} = 3.48$, P
118 $= 0.08$) or interaction between the two fixed effects (LMM: $F_{1,18} = 0.05$, $P = 0.82$). This model
119 explains 99.31% of chromatic contrast variance, 96.50% of which is explained by adjacency of
120 the color elements, while changes in horizontal stripe luminance explain the remaining variance.
121 On the other hand, we found that achromatic contrasts are strongly influenced by changes in
122 luminance of the horizontal stripe (LMM: $F_{1,18} = 9.11$, $P = 0.007$), as the balance between
123 adjacent and non-adjacent contrasts (LMM: $F_{1,18} = 5.07$, $P = 0.037$) and the interaction between
124 the two becomes important (LMM: $F_{1,18} = 6.78$, $P = 0.018$; empty circles in Figure 3D and S1I).
125 This model explains 95.90% of the achromatic contrast variance, 68.53% of which is explained
126 by changes in darkness of the horizontal stripe, 22.34% by signal design (patch adjacency) and
127 the remainder 5.02% by their interaction. Thus, we find that white, yellow and blue are less
128 dynamic elements of the facial color pattern, and seem to provide little or no information
129 regarding changes in aggressive intent but instead act as amplifiers to enhance pattern
130 conspicuousness.

131 Our visual models and behavioral experiments indicate that individuals use rapid
132 physiological changes in luminance (achromatic contrast) of the horizontal stripe element to
133 dynamically communicate reversals in aggressive intent and dominance, while the color pattern
134 conspicuousness remains high at all times by virtue of its stable chromatic properties. Using this
135 dual mechanism is an elegant way to ensure that conspicuousness, and hence communication
136 efficacy, does not decrease due to context-dependent signaling. This constantly 'on' signaling
137 strategy of aggressive intent in *N. brichardi* is unexpected as it is opposite to other signaling

138 systems such as in anoles lizards, chameleons, or transiently territorial fish which only briefly or
139 seasonally display their signals during agonistic encounters [32, 34–36] (Figure 2). Our findings
140 could possibly be explained by lifelong territoriality, constant interactions with conspecifics and
141 different predation escape strategies of *N. brichardi*. While these stenotopic cichlids rely on their
142 valuable rocky territories for shelter (and breeding) and conspicuously signal their ownership at
143 all times, chameleons have to rely on immobility and camouflage to escape avian predation and
144 become only momentarily conspicuous while displaying to conspecifics [35]. Instead, from a
145 signaling perspective uninterrupted conspicuousness of *N. brichardi* is more similar to that of
146 aposematic species, which rely on high conspicuousness to continuously signal their
147 distastefulness [37, 38].

148

149 **Stage 3: Proximate mechanisms producing an evolutionary stable signaling strategy.** Using
150 a dyadic combat experiment in combination with the visual models we showed that changes in
151 luminance of the horizontal melanistic stripe are used during social agonistic interactions and
152 correlate with aggressive intent. However, whether the fish directly respond to the physiological
153 color changes of the horizontal facial stripe and if these changes are then used to assess another
154 individual's aggressive intentions needs direct behavioral evaluation. Moreover, if changes in
155 luminance were to reliably signal aggressive intent, we would expect the existence of an honesty
156 mechanism to minimize cheating opportunities. To test these expectations we simulated a cheater
157 invasion of the signaling system, by manipulating luminance of the horizontal facial stripe out-
158 of-equilibrium (via artificial darkening or paling; Figures S2C, S2D and Table S1) and
159 presenting fish to their mirror images. Our setup is opposite to the commonly used approach of
160 displaying manipulated individuals to focal territory owners as noted by Bradbury and

161 Vehrencamp [3], having the advantage of testing behavior of non-territorials (i.e. the receivers of
162 the mirror image), which are the ones most interested in detecting unreliable signals if used by
163 territorial, dominant individuals. As a null hypothesis (Figure 1), (1) we do not expect to observe
164 differences in aggression toward manipulated or non-manipulated individuals if stripe intensity
165 does not encode individual fighting abilities (but simply correlates with them). On the other
166 hand, (2) if stripe intensity signals a contest-independent intrinsic quality whereby strategic costs
167 guarantee honesty (e.g. handicap), subordinates should not challenge cheating individuals with
168 enhanced signals but should do so toward individuals with subdued signals. Alternatively, (3) if
169 stripe intensity signals contest-dependent dominance whereby social costs (i.e. punishment of
170 cheaters) maintain signal honesty, we expect increased levels of aggression toward any
171 unreliable signal (i.e. a conventional signal).

172 We found that receivers actively ‘read’ and react to manipulations of the horizontal facial
173 stripe, recognizing and punishing cheaters (Figures 4B and S2E-G). Manipulation of the
174 horizontal stripe had a significant effect on the number of aggressive bouts received (LMM: $F_{2,45}$
175 = 13.73, $P < 0.001$), irrespective of sex (LMM: $F_{2,45} = 0.48$, $P = 0.62$). Individuals with darkened
176 stripes received significantly more aggression than individuals with paled stripes (Tukey HSD: z
177 = -3.89, $P < 0.001$) and controls (Tukey HSD: $z = -6.59$, $P < 0.001$). Importantly, individuals
178 with paled stripes also received more aggression than controls (Tukey HSD: $z = -2.97$, $P =$
179 0.008), indicating that unreliable signaling brings increased marginal costs to all types of
180 cheaters.

181 By manipulating the signal out-of-equilibrium we simultaneously show that physiological
182 color changes are interpreted by receivers as a dynamic context-dependent signal of aggressive
183 intent and that social selection by receiver retaliation is the proximate mechanism effectively

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

199 Physiological color changes have previously been implicated in signaling aggressive
200 intent in a number of taxa, in particular fish [32, 41–43]. Increased levels of aggression toward
201 the signal reported in some of these studies were interpreted as receiver retaliation costs
202 maintaining honesty of a conventional signal. We extend these findings and show that unreliable
203 signaling has increased costs relative to reliable signaling, which is pivotal to the evolution of
204 honest signals [2, 13, 17]. The rapid physiological color changes of this dynamic color signal
205 rival the morphological and behavioral context-dependent signaling strategies evolved by other
206 territorial species [44, 45] and allow the fish to instantaneously communicate their intention to

207 fight or retreat from the combat by reliably showing or concealing the signal of aggressive intent.
208 Such dynamic expression noted among fish [46] contrasts with other more or less static visual
209 signals, such as plumage badges of status in birds [47, 48], exoskeleton color patterns in insects
210 [31], or aposematic signals in poison frogs [37], all of which are thought to signal more
211 temporally stable characteristics of quality and distastefulness.

212 In summary, our framework for color signal analysis proved important in generating
213 predictions according to trends emerging from theory. We demonstrated that the facial mask of
214 *N. brichardi* has stable chromatic properties that keep signaling efficacy high at all times, while
215 rapid physiological changes in luminance of just one element (the horizontal melanic stripe)
216 communicate reversals in aggressive intent and dominance. We further demonstrated that social
217 selection maintains honesty of the signaling system, which could be nature's favored mechanism
218 for promoting the honesty of dynamic visual signals with low production costs, such as those
219 produced by physiological color changes. Together, these findings suggest that social selection
220 may account for the dramatic diversity of color patterns (stripes, bars, blotches) we observe in
221 many sexually monochromatic cichlid species endemic to Lake Tanganyika [22] and elsewhere,
222 acting together with natural selection in shaping diversity in cichlid fishes [49, 50]. Social
223 selection is expected to drive rapid signal evolution especially in isolated allopatric populations
224 [14, 51], but until now research into color signaling in cichlids has centered on the sexually
225 dichromatic assemblages from Lake Malawi and Lake Victoria [19]. Our results point to rapid
226 social trait evolution as another process potentially affecting speciation dynamics in cichlids.
227 Confirmation of its importance would bring social selection to the same level as sexual and
228 natural selection in shaping adaptive radiations of cichlid fishes.

229

230 **AUTHOR CONTRIBUTIONS**

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

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

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

235

236 **ACKNOWLEDGMENTS**

237 This work was supported by the Swiss National Science Foundation (SNSF) grant 138224 to

238 WS. Photographs of the anole and widowbird are a courtesy of Melisa Losos and Jan Willem

239 Steffelaar. We thank designers Inês Santiago and Marco Silva for comments on figure

240 preparation, and Adrian Indermaur for fishkeeping.

241

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350

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

354

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

364

365 **Figure 3. Color properties of facial elements in dominant and subordinate *Neolamprologus***
366 ***brichardi***

367 (A and B) Mean spectral reflectance of facial color patches. (A) Horizontal (green triangle) and
368 vertical (black triangle) facial stripes have the same reflectance in dominant fish (note arrow).
369 (B) Losing a combat and becoming subordinate significantly increases reflectance of horizontal
370 facial stripe in subordinate fish, i.e. paling occurs. (See Figure S1G for 95% confidence intervals
371 of spectral reflectance).
372 (C and D) Chromatic and achromatic contrasts (mean \pm SEM) between pairs of adjacent and
373 non-adjacent color patches as perceived by *N. brichardi*, ordered from highest to lowest in
374 dominant fish. (C) High chromatic contrast ΔS is achieved by any combination of blue, yellow
375 and black patches. (D) High achromatic contrast ΔL is achieved by combining black melanic
376 stripes and other patches. Stippled line marks the 1 JND (just noticeable difference), threshold
377 after which two colors are thought to be perceived as different [29, 53]. Asterisks illustrate
378 significant differences in contrast between dominant and subordinate fish (*** $P < 0.001$, ** $P <$
379 0.01 , * $P < 0.05$). (Figure S1 shows data used to build visual models)

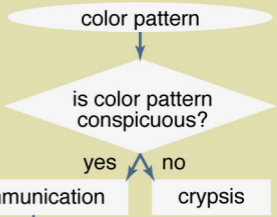
380

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

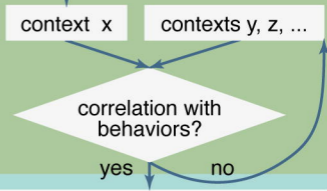
382 (A) Facial stripe intensity is associated with fighting ability (winning or losing) at end of combat.
383 L: losers; W: winners.

384 (B) Social selection (aggressive bouts, mean \pm SEM) on out-of-equilibrium signals and control.
385 Unreliable signaling of strength (darkened stripe) and weakness (paled stripe) are punished by
386 increased receiver retaliation costs relative to reliable signaling (control). Asterisks illustrate
387 significant differences in facial stripe luminance at end of combat and of pairwise post-hoc tests
388 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

