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: $\chi_1^2 = 14.97$, $P < 0.001$; 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: $\chi_1^2 = 0.01$, 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

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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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

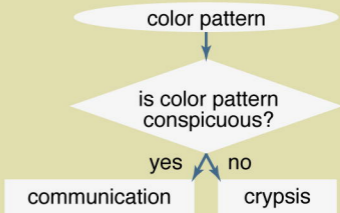363   epaulette in Fan-tailed Widowbird *(Euplectes axillaris)*.

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 ± 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 (*** $P < 0.001$, ** $P <$

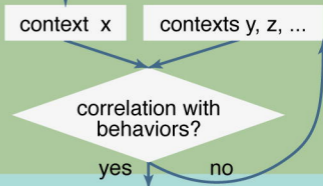379   0.01, * $P < 0.05$). (Figure S1 shows data used to build visual models)

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 ± 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).
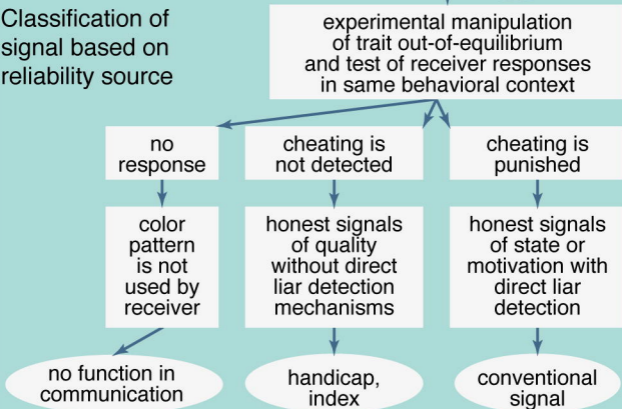
Characterization of trait properties relative to visual system of receiver

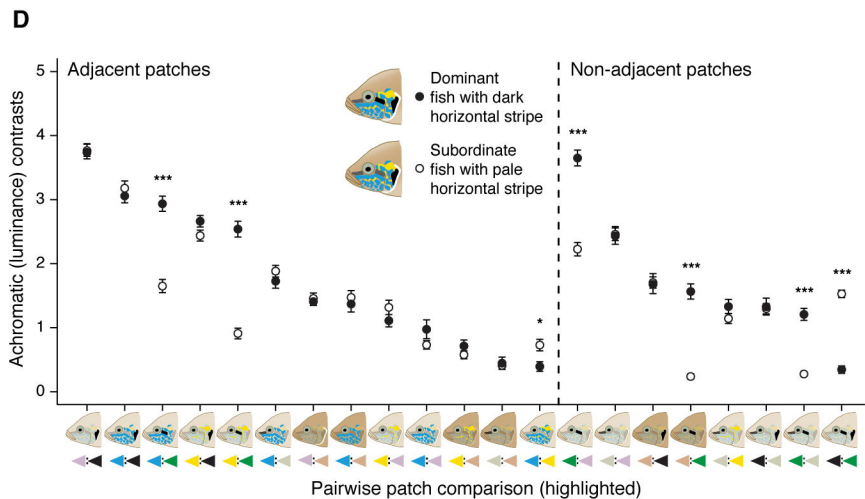color pattern

↓

is color pattern conspicuous?

yes / no

communication | crypsis

Determination of message encoded

context x | contexts y, z, ...

↓

correlation with behaviors?

yes / no

Classification of signal based on reliability source

experimental manipulation of trait out-of-equilibrium and test of receiver responses in same behavioral context

no response | cheating is not detected | cheating is punished

↓

color pattern is not used by receiver | honest signals of quality without direct liar detection mechanisms | honest signals of state or motivation with direct liar detection

↓

no function in communication | handicap, index | conventional signal

A Dominant fish with dark horizontal stripe

Spectral reflectance (%) vs Wavelength (nm)

B Subordinate fish with pale horizontal stripe

Spectral reflectance (%) vs Wavelength (nm)

C

Chromatic (hue) contrasts

Adjacent patches | Non-adjacent patches

Dominant fish with dark horizontal stripe
Subordinate fidh with pale horizontal stripe

Pairwise patch comparison (highlighted)

D

Achromatic (luminance) contrasts

Adjacent patches | Non-adjacent patches

Dominant fish with dark horizontal stripe
Subordinate fish with pale horizontal stripe

Pairwise patch comparison (highlighted)

A

Loser

Winner

No. individuals

■ Dark horizontal stripe ■ Pale horizontal stripe

***

B

Aggressive bouts

Darkened   Paled   Control

*** ** ***