## SUPPLEMENTARY MATERIAL FOR:

## Social costs enforce honesty of a dynamic signal of motivation

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## **Blind data collection**

Observers quantified numerous agonistic behaviors (Table S2) from video recordings which were focused on the unpainted chameleons. A separate camera was focused on the painted chameleon in each trial, allowing their behavior to be independently categorized as aggressive or non-aggressive. The filming and behavioral quantification procedures typically prevented observers from detecting the treatment (painted dark or bright) or behavior (aggressive or not) of the painted chameleons (unless the chameleons came into direct contact). Even in cases of direct contact, however, observers did not necessarily know the behavioral category of the opponent (aggressive vs. not) and could not therefore know whether they fit into honest or dishonest categories. Further, the conceptual background provided to the trained observers simply framed the study as one designed to investigate the relative importance of color and behavior, not as a test of social costs associated with dishonest signaling, further reducing the likelihood of bias.

Cone ratio	Color comparison	Chromatic JNDs	Achromatic JNDs§
UV:SWS:MWS:LWS			
1:1:1:1			
	Brown	4.97	4.01
	White	2.80	2.69
	Yellow	4.61	1.63
	Green	5.57	1.03
	Orange	4.97	3.62
	Blue-green	4.62	2.08
2:3:15:75			
	Brown	1.88	
	White	0.80	
	Yellow	0.92	
	Green	1.13	
	Orange	1.26	
	Blue-green	1.18	
1:1:3:6			
	Brown	3.17	
	White	1.56	
	Yellow	2.08	
	Green	2.64	
	Orange	2.53	
	Blue-green	2.30	

**Table S1**. Influence of varying ratios of different cone types in chameleon eyes on calculated perceptual distances between custom paints and exemplar chameleon skin colors. Cone ratios influence noise-to-signal ratios incorporated into visual models [1,2]. Cone ratios used to determine perceptual distinctiveness between custom paints and chameleon colors vary from 1:1:1:1 ratio (frequently used when data concerning relative abundance of cone types is lacking) to 2:3:15:75 (obtained from counts in reference [3]).

<sup>§</sup> Luminance detection is process of double-cone stimulation in reptiles, which have the same peak sensitivities as the long-wavelength sensitive cones [3]. Achromatic contrasts are a function of differential stimulation of the double cones [4] and, consequently, do not change with varying ratios of the other cone types.

Behav	ioral metric	Description	
Numb	er of		
	Fights <sup>†</sup>	Physical contact and intent to bite or displace opponent	
behaviors	Knock-offs <sup>†</sup>	Chameleon aggressively dislodges opponent from perch	
	Attacks <sup>†</sup>	Initiation of physical contact	
	Bite-releases <sup>†</sup>	Biting followed by immediate release of opponent	
	Bite-clamps <sup>†</sup>	Sustained biting (locked on to opponent with mouth)	
sive	Approaches <sup>†</sup>	Directed movement towards opponent	
res	Lunges <sup>†</sup>	Fast, directed head or body thrust towards opponent	
Submissive Agg behaviors	Lateral displays <sup>§</sup>	Lateral compression, dorso-ventral expansion, physical orienting of body perpendicularly to opponent	
	Swaying bouts <sup>§</sup>	Lateral, side-to-side movement of entire body	
	Tail curls <sup>§</sup>	Tail curled and uncurled	
	Retreats	Directed movement away from opponent	
	Times fleeing	Rapid, directed movement way from opponent	
Comp	osite scores		
I	Physical aggression	Summed total of physical aggressive behaviors	
I	Aggressive displays	Summed total of non-physical aggressive behaviors	
Did th	is behavior occur? (Yes/No)		
Approach?		Did the chameleon approach?	

**Table S2**. Behavioral metrics quantified to evaluate inter-observer reliability. The number of physical and non-physical aggressive behaviors exhibited by non-painted chameleons was used as to quantify the aggression received by painted chameleons.

**Table S3.** Overall aggression exhibited by chameleons is strongly predicted by whether they approached their opponents. Specifically, cumulative aggression (sum of all aggressive behaviors, not including approaches) was predicted by approach behavior using a GLMM with Poisson error structure and including chameleon ID as a random effect. Estimate = slope estimate; s.e. = standard error;  $R_m^2$  = marginal  $R^2$ , corresponding to variance explained by fixed effects;  $R_c^2$  = conditional  $R^2$ , corresponding to variance explained by fixed and random effects.

Model	Fixed effect	Estimate	s.e	Z	р
Cumulat	ive aggression exhibited (not including approach)				
$(R_m^2=0.$	58, $R_c^2 = 0.66$ )				
	Intercept	0.24	0.31	0.75	0.451
	Approached opponent? (yes/no)	2.33	0.37	6.33	< 0.0001

**Table S4.** Aggressive (non-physical) displays directed towards painted chameleons. When painted chameleons are categorized as aggressive or not aggressive, their aggression and paint treatment influence the displays directed towards them, but the interaction between these two variables is not significant. Parameters calculated using GLMM with painted chameleon identity included as random effect and Poisson distribution. Estimate = slope estimate; s.e. = standard error;  $R_m^2$  = marginal  $R^2$ , corresponding to variance explained by fixed effects;  $R_c^2$  = conditional  $R^2$ , corresponding to variance explained by fixed effects.

Model	Fixed effect	Estimate	s.e	Z	р
Non-phys	ical aggression received				
$(R_m^2 = 0.5)$	7, $R_c^2 = 0.79$ )				
	Intercept	2.00	0.23	8.53	< 0.0001
	Treatment (painted bright/dark)	0.43	0.22	1.95	0.052
	Aggressive (yes/no)	-1.92	0.54	-3.53	< 0.001
	Treatment* Aggression	-0.43	0.50	-0.86	0.388



**Figure S1.** Artificial paints used to manipulate chameleon color signals matched exemplar chameleon skin color. (a-f) Reflectance curves of artificial paints (dashed lines) closely matched exemplar chameleon colors (solid lines) for six different colors (a – yellow, b – orange, c – green, d – blue-green, e – white, f - brown).





Figure S2. Normalized spectral sensitivities of chameleon cones used to model perceptual match between custom paints and real chameleon colors. Chameleons have four classes of cones, ultra-violet sensitive (UVS), short-wavelength sensitive (SWS), medium wavelength sensitive (MWS), and long wavelength sensitive (LWS) cones [45]. Normalized absorbance/sensitivity illustrated here reflects spectral sensitivity of the individual cone types while accounting for the light transmission properties of the lens, cornea, and oil droplets [45].



Figure S3. Normalized irradiance of the experimental trial arenas in which chameleons faced one another in dyadic encounters.



**Figure S4.** Non-physical aggression received by painted chameleons was influenced by paint treatment and the aggressive behavior of painted chameleons, but not the interaction between these variables. Bar heights indicate mean values ( $\pm$  SEM), sample sizes are indicated above each bar, and model statistics for relevant analyses are located in Table S4.

## Literature Cited in Supplementary Material

- Vorobyev, M. & Osorio, D. 1998 Receptor noise as a determinant of colour thresholds.
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