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Appendix from M. E. Maan and M. E. Cummings, "Poison Frog Colors Are Honest Signals of Toxicity, Particularly for Bird Predators"

(Am. Nat., vol. 179, no. 1, p. E1)

Supplementary Methods and Results



Figure A1: Light environments used for estimating color and brightness contrast. *A*, Irradiance in the forest shade. *B*, Reflectance spectra for three natural background substrates: green *Heliconia*, brown leaf litter, and black-brown tree bark.

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Figure A2: Relationship between *Dendrobates pumilio* toxicity score and viewer-independent coloration brightness (total reflectance flux; arbitrary units). *A*, Populations with brighter dorsal coloration are more toxic. *B*, There is no significant relationship between ventral coloration brightness and toxicity. Numbers refer to the population labels in figure 2.

5 ē Ξ 1,0 1,0 1,0 ●3_●2 ●3_{●2} L) background: *Heliconia* $\bullet^3 \bullet^2$ R²=0.003, p=0.88 0,8 0,8 0,8 snake viewer K²=0.003, p=0.88 toxicity score 0,6 0,6 J) background: bark R²=0.003, p=0.89 0,6 00 e ●10●9 ●8 0,4 0,4 0,4 ° 000 €00 €**1**0**9** 0,2 0,2 0,2 1,0 1,0 1,0 0,9 0,8 0,7 0,6 0,9 0,8 0,7 0,6 0,9 0,8 0,7 0,6 ē ē ē 1,0 1,0 1,0 ●3_{●2} ●³●2 .H) background: leaf litter $\bullet^3 \bullet^2$ R²=0.006, p=0.84 0,8 0,8 0,8 I) background: Heliconia toxicity score crab viewer 4 0 20 G) background: bark R²=0.001, p=0.94 0,6 0,6 0,6 R²=0.008, p=0.80 0 0 ●10●9 ●8 ●10●9_{●8} ●10_{●9_{●8}} 0,4 6 0,4 0,4 0,2 0,2 0,2 1,0 0,8 0,6 0,2 0,0 1,0 0,8 0,6 0,2 0,0 1,0 0,8 0,6 0,0 0,4 0,4 0,4 0,2 ē ē ē 1,0 1,0 1,0 •3_{•2} •3 •2 •3 •2 0,8 0,8 0,8 F) background: Heliconia toxicity score ⁻E) background: leaf litter R²=0.058, p=0.50 bird viewer 4 4 D) background: bark R²=0.044, p=0.56 0,6 0,6 0,6 R²=0.044, p=0.56 0,4 0,4 0,4 5 5 0,2 0,2 0,2 1,0 0,8 0,6 0,4 0,2 0,0 1,0 0,8 0,6 0,4 0,2 0,0 1,0 0,8 0,6 0,4 0,2 0,0 ē ē ē 1,0 1,0 1,0 ●3_{●2} •3_{•2} •3_{•2} D. pumilio viewer 0,8 0,8 0,8 C) background: *Heliconia* R²=0.002, p=0.91 toxicity score B) background: leaf litter R^2 =0.001, p=0.93 • 4 A) background: bark R²=0.001, p=0.95 0,6 0,6 0,6 90 00 0,4 0,4 0,4 8 ●10●9 0,2 0,2 0,2 1,0 0,9 0,8 0,7 0,6 1,0 0,9 0,8 0,7 0,6 1,0 0,9 0,8 0,7 0,6 overall conspicuousness overall conspicuousness overall conspicuousness





Figure A4: Frog toxicity in relation to the luminance contrast of dorsal coloration, as estimated for four different viewers and three different background substrates (labels as in fig. A3). Solid lines are statistically significant relationships; broken lines indicate statistical trends. Each symbol represents one *Dendrobates pumilio* population, where numbers refer to the population labels in figure 2.



Figure A5: Frog toxicity in relation to the spectral contrast of dorsal coloration, as estimated for four different viewers and three different background substrates (labels as in fig. A3). Solid lines are statistically significant relationships. Each symbol represents one *Dendrobates pumilio* population, where numbers refer to the population labels in figure 2.



Figure A6: Frog toxicity in relation to the luminance contrast of ventral coloration, as estimated for four different viewers and three different background substrates (labels as in fig. A3). Each symbol represents one Dendrobates pumilio population, where numbers refer to the population labels in figure 2.





Coloration measure	F _{3, 105}	Р
Differences between viewers, controlling for background substrate:		
Dorsal luminance contrast (ΔL)	32.56	<.001
Dorsal spectral contrast (ΔS)	50.66	<.001
Dorsal overall conspicuousness	41.15	<.001
Ventral luminance contrast (ΔL)	23.71	<.001
Ventral spectral contrast (ΔS)	30.32	<.001
Ventral overall conspicuousness	20.88	<.001
ventar overan conspicatousness		Р
Differences between background substrates, controlling for viewer:		
Dorsal luminance contrast (ΔL)	.301	.74
Dorsal spectral contrast (ΔS)	.334	.72
Dorsal overall conspicuousness	.456	.64
Ventral luminance contrast (ΔL)	3.807	.025
Ventral spectral contrast (ΔS)	.213	.81
Ventral overall conspicuousness	1.047	.35

Table A1. Test results for the differences in conspicuousness estimates between different viewers and different background substrates for all coloration measures

Table A2. Pearson correlations between frog toxicity and dorsal coloration (spectral contrast $[\Delta S]$ and overall conspicuousness) as perceived by two alternative bird visual systems, UV and UVS

	UVS		VS	
Coloration measure and background	r	Р	r	Р
Spectral contrast (ΔS):				
Bark	.54	.11	.52	.13
Heliconia	.68	.032	.68	.030
Leaf litter	.45	.19	.45	.20
Overall conspicuousness:				
Bark	.85	.0017	.85	.0020
Heliconia	.97	<.001	.97	<.001
Leaf litter	.93	<.001	.93	<.001

Note: In all reported results, we estimated frog conspicuousness as viewed by birds using a UVS visual model. For bird perception of frog dorsal coloration, we also evaluated conspicuousness using a VS visual model (based on the pigeon, *Columba livia*, $\lambda_{max} = 409$ nm; corrected for screening pigments [after Bowmaker et al. 1997]). This did not affect the estimates of luminance contrast (ΔL) but yielded slightly different estimates of spectral contrast (ΔS) and thereby also overall conspicuousness. However, the differences were very small and far from statistically significant; ΔS : $F_{2,47} = 0.062$, P = .80; overall conspicuousness: $F_{2,47} = 0.0024$, P = .96. The correlations with frog toxicity did not change qualitatively. Significant correlations are in bold.

Table A3. Test results for the difference between species-specific or fixed ($\omega = 0.12$) noise levels for estimated spectral contrast and overall conspicuousness, controlling for background substrate

e			
Viewer and coloration measure	$F_{2, 47}$	Р	
Bird (UVS):			
Spectral contrast (ΔS)	17.13	<.001	
Overall conspicuousness	4.97	.031	
Frog:			
Spectral contrast (ΔS)	109.01	<.001	
Overall conspicuousness	94.95	<.001	
Snake:			
Spectral contrast (ΔS)	54.51	<.001	
Overall conspicuousness	119.86	<.001	

Table A4. Pearson's correlation coefficients and *P* values for the relationships between frog toxicity and estimates of spectral contrast and overall conspicuousness, for visual models with species-specific noise levels and for models with fixed noise levels ($\omega = 0.12$)

	Noise estimates			
Viewer, measure, and background	Species specific		Fixed	
	r	Р	r	Р
Bird (UVS):				
Spectral contrast (ΔS):				
Bark	.52	.13	011	.98
Heliconia	.68	.030	.84	.002
Leaf litter	.45	.20	.38	.28
Overall conspicuousness:				
Bark	.85	.002	.93	<.001
Heliconia	.97	<.001	.96	<.001
Leaf litter	.93	<.001	.85	.002
Frog:				
Spectral contrast (ΔS):				
Bark	.53	.11	.50	.14
Heliconia	.53	.11	.50	.14
Leaf litter	.53	.11	.50	.14
Overall conspicuousness:				
Bark	.61	.060	.61	.062
Heliconia	.62	.054	.61	.060
Leaf litter	.62	.057	.61	.061
Snake:				
Spectral contrast (ΔS):				
Bark	.41	.24	.48	.16
Heliconia	.41	.24	.50	.14
Leaf litter	.41	.24	.49	.15
Overall conspicuousness:				
Bark	.51	.13	.51	.13
Heliconia	.51	.13	.52	.13
Leaf litter	.51	.13	.52	.13

Note: Significant correlations are in bold; statistical trends are in italics.

Table A5. Test results evaluating the effect of fixed noise levels ($\omega = 0.12$) on the relationship between frog toxicity and visual conspicuousness

Viewer, measure, and background	$F_{2, 17}$	Р
Bird (UVS):		
Spectral contrast (ΔS):		
Bark	4.29	.054
Heliconia	1.11	.31
Leaf litter	2.32	.15
Overall conspicuousness:		
Bark	3.22	.091
Heliconia	.033	.86
Leaf litter	1.10	.31
Frog:		
Spectral contrast (ΔS):		
Bark	.24	.63
Heliconia	.26	.62
Leaf litter	.25	.62
Overall conspicuousness:		
Bark	.39	.54
Heliconia	1.02	.33
Leaf litter	.73	.40
Snake:		
Spectral contrast (ΔS):		
Bark	.24	.63
Heliconia	.53	.47
Leaf litter	.41	.53
Overall conspicuousness:		
Bark	.69	.42
Heliconia	2.48	.13
Leaf litter	1.60	.22

Note: In all reported results, we estimated species-specific noise levels using reported taxon-specific Weber fractions and photoreceptor cone class proportions (except for the crab visual system, where we used honeybee physiological measurements of noise levels fixed at $\omega = 0.12$ for each cone class). To evaluate how robust our results are with respect to noise settings, we also calculated conspicuousness estimates for dorsal coloration for the other three viewers (frog, bird, snake) assuming this fixed noise level. This yielded significantly different estimates of spectral contrast (ΔS) and overall conspicuousness for each of the three viewer taxa (table A3). For the correlations with frog toxicity, however, fixed noise levels did not make much difference: all significant results and statistical trends remained (table A4). We then used glm and ANOVA to formally evaluate the consequence of fixed noise levels on the relationship between toxicity and conspicuousness (table A5). This analysis revealed two statistical trends, indicating a change in the strength of the relationship between toxicity and coloration spectral contrast and overall conspicuousness, as viewed by birds. However, both these relationships remained highly significant (see table A4). Statistical trends are in italics.

		All		Without	
Viewer measure and background	r	P	r	p	
		1			
Viewer-independent brightness (total reflectance flux ΣR)	.78	.0078	.73	.025	
Viewer- and background-specific estimates of coloration:					
Bird (UVS):					
Luminance contrast (ΔL):					
Bark	.82	.00	.75	.02	
Heliconia	.96	.00	.94	.00	
Leaf litter	.93	.00	.90	.00	
Spectral contrast (ΔS):					
Bark	.52	.13	.18	.65	
Heliconia	.68	.03	.48	.19	
Leaf litter	.45	.20	10	.79	
Overall conspicuousness:					
Bark	.85	.00	.79	.01	
Heliconia	.97	.00	.96	.00	
Leaf litter	.93	.00	.90	.00	
Crab:					
Luminance contrast (ΔL):					
Bark	.52	.12	.56	.12	
Heliconia	.20	.58	.59	.10	
Leaf litter	.58	.08	.71	.03	
Spectral contrast (ΔS):					
Bark	30	.40	20	.60	
Heliconia	.71	.02	.61	.08	
Leaf litter	.66	.04	.58	.10	
Overall conspicuousness:					
Bark	60	06	.70	.04	
Heliconia	.00	20	62	.07	
Leaf litter	.64	.05	.02	.02	
Frog		100	•/ •	.02	
I uminance contrast (ΛI) :					
Bark	58	08	58	10	
Heliconia	.50	.00	58	10	
Leef litter	.50	.00	.50	.10	
Separate l contract (AS)	.50	.00	.58	.10	
Spectral contrast (ΔS):	52	11	22	5(
Bark Halisanin	.33	.11	.22	.30	
Henconia	.55	.11	.22	.30	
	.55	.11	.22	.50	
Overall conspicuousness:	(1	06			
Bark	.61	.06	.57	.11	
Heliconia	.62	.05	.56	.12	
Leaf litter	.62	.06	.56	.11	
Snake:					
Luminance contrast (ΔL):					
Bark	.51	.13	.53	.14	
Heliconia	.51	.13	.53	.14	
Leaf litter	.51	.13	.53	.14	
Spectral contrast (ΔS):					
Bark	.41	.24	.18	.64	
Heliconia	.41	.24	.19	.62	
Leaf litter	.41	.24	.19	.63	
Overall conspicuousness:					
Bark	.51	.13	.53	.14	
Heliconia	.51	.13	.53	.14	
Leaf litter	.51	.13	.53	.14	

 Table A6.
 Pearson correlations between frog toxicity and coloration, with and without the Solarte population

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Note: Our toxicity estimate for the Solarte population was much higher than that of Daly and Myers (1967), while estimates for the other populations were in line with that study. Because Solarte frogs are also the most conspicuous, this population may strongly influence the overall pattern. To evaluate the robustness of our main result, we therefore recalculated correlations between frog toxicity and dorsal conspicuousness excluding the Solarte population. We found that the overall positive and significant relationships remain. Significant correlations are in bold; statistical trends are in italics.