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Supporting information for

Bioluminescent backlighting illuminates the complex visual signals of a social squid in the deep sea

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SI Methods

To visualize the phylogenetic context of the proposed mechanism by which pigmentation patterns can function as visual signals in darkness, we mapped relevant ecological, morphological, and behavioral information to a published genus-level phylogeny of the Decabrachia (squids and cuttlefish) (1) (Figure S3). Reference (1) conducted phylogenetic inference using a partitioned maximum likelihood analysis of publicly available mitochondrial and nuclear marker sequences with 1000 bootstrap replicates under the GTR model of evolution with Gamma distribution (*Vampyroteuthis* as the outgroup). This phylogeny includes the largest taxonomic coverage and collection of marker sequences to date, is generally congruent with previous work (2-5), and supports major clades including the Ommastrephinae. Lower typical daytime depth distributions of genera included in the phylogeny were determined from published depth ranges (6, 7), of all species within each genus (if known), and categorized by the predominant mode within a genus: mesopelagic (200-1000 m) and deeper, or epipelagic (0-200). If species were evenly split between these categories, then the genus was assigned to the mesopelagic and epipelagic category. Possession of numerous, small subcutaneous photophores within each genus was determined from published morphological information (6, 7), and we compiled the relevant literature to ascertain the chromatic component repertoires (8-25). The number of chromatic components displayed by a genus was categorized as many (>10) or few (≤10) based on the average component repertoire of all species studied within a genus.

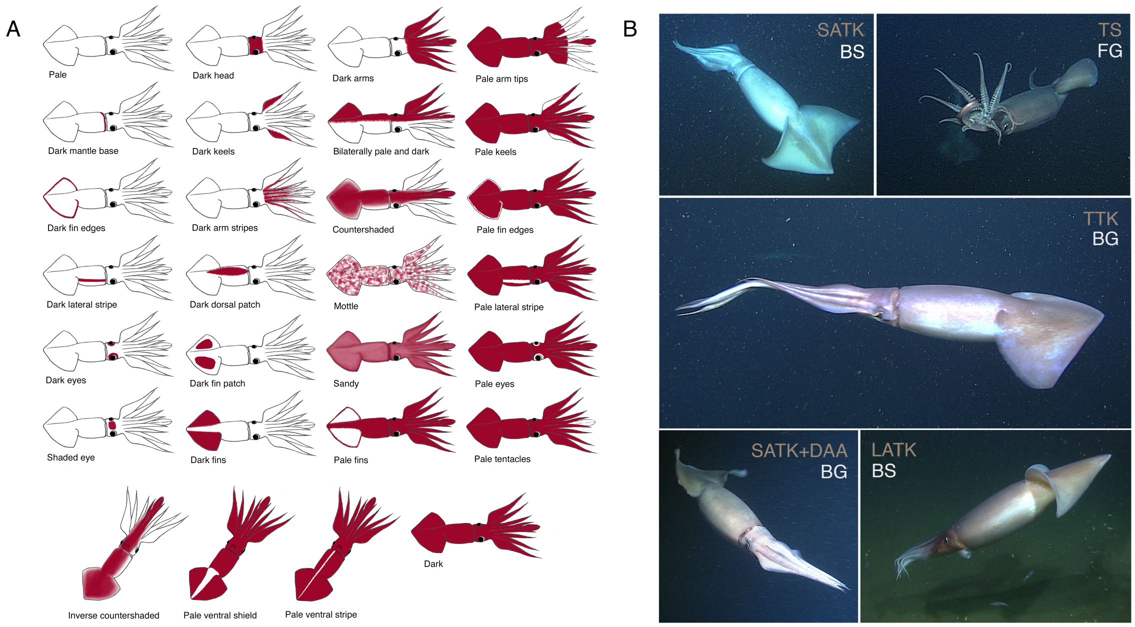

Figure S1.

A) Illustrations of the 28 chromatic components performed by *Dosidicus gigas* (related to Figure 1-3). *Inverse countershaded*, *pale ventral shield*, and *pale ventral stripe* (bottom row) are drawn on squid with their ventral surface facing up. B) Examples of various postural and locomotor components analyzed in this study (related to Figure 2-3). See (Table 1) for component abbreviations.

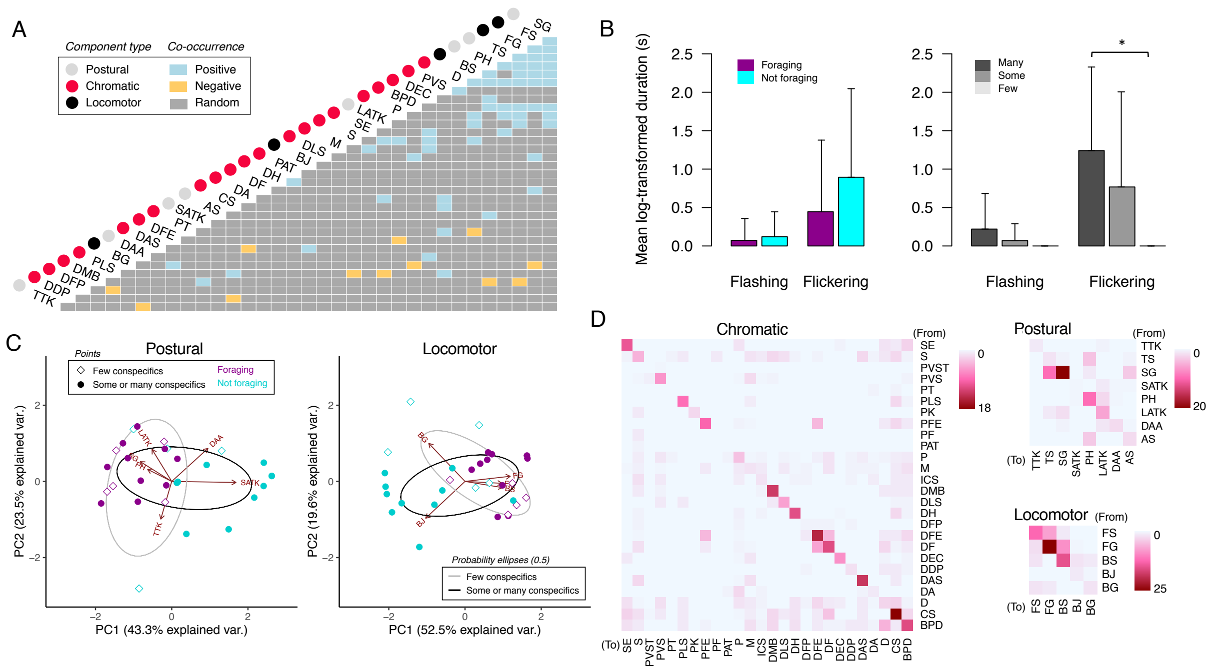

Figure S2.

A) Positive, negative, and random component associations of *D. gigas* (n=30) determined by a probabilistic co-occurrence model (related to Figure 1-2). Component abbreviations indicate the columns and rows representing their pairwise relationships with other components. Point color indicates component type, and box color represents co-occurrence category. B) Comparison of the average log-transformed duration of variable chromatic components (*flashing* and *flickering*) between foraging status and conspecific abundance categories in *D. gigas* (n=30 each). Bars represent standard deviation of the mean. *Significant difference between means (ANOVA, Tukey HSD) (see Table S2). In this study, foraging and not foraging distinguish whether or not a squid attempted to capture prey. C) Principal component analyses and vectors of postural and locomotor components with 50% probability ellipses encircling conspecific abundance clusters (related to Figure 2). In both, each *D. gigas* (n=30) is represented by a single point colored by foraging status and shaped by conspecific abundance category. Red arrows and abbreviations represent the vectors, or relative contribution of different components to the behavioral trends among squid. D) Adjacency network heatmaps of chromatic, postural, and locomotor components displayed by foraging *D. gigas* (n=15) (related to Figure 2). Color denotes the number of occurrences that squid transitioned from components on the vertical axes to components on the horizontal axes, with prolonged single-component display included. See (Table 1) for component abbreviations.

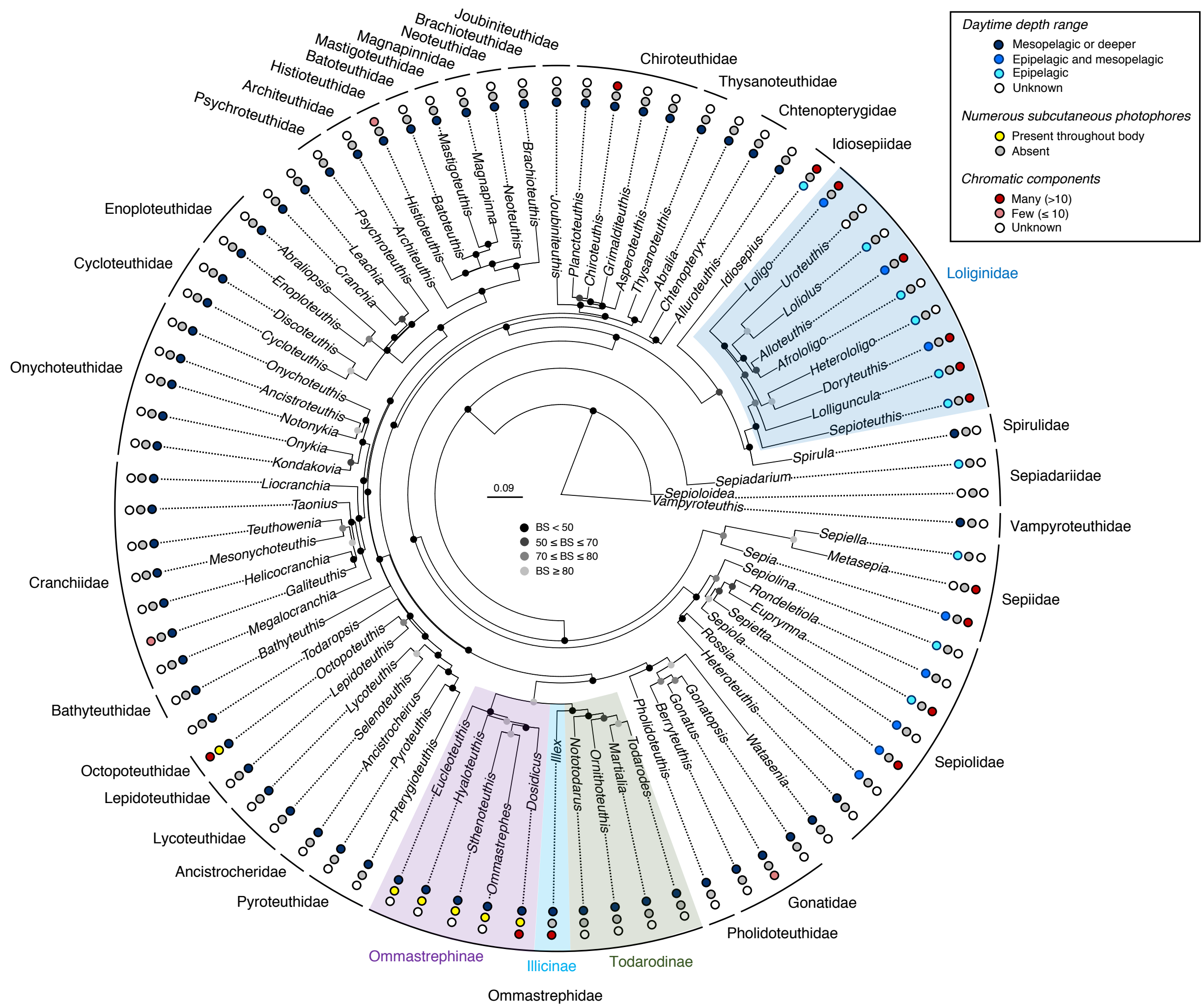

Figure S3.

Phylogenetic context of the proposed mechanism by which pigmentation patterns can function as visual signals under low-light conditions (related to Figure 3). Genus-level phylogeny of the Decabrachia (squids and cuttlefish) from reference (1) overlaid with relevant ecological, morphological, and behavioral information. Bootstrap support for each clade is color-coded with points, and four clades are shaded: subfamily Ommastrephinae (purple), Illicinae (light blue), and Todarodinae (green), and family Loliginidae (blue). Note that all genera in the Ommastrephinae have deep daytime depths and numerous subcutaneous photophores.

Component	Description	Eigenvectors	
Postural		*PC1 (43.3%, 1.95)*	*PC2 (23.5%, 1.06)*
Dorsal arm arch	Slight raising of first arm pair while all arms together and pointed ("Dorsal arm lift," †)	0.42	-0.52
Loose arm tips, keeled	Arms bundled posteriorly and loose anteriorly, third arm aboral extensions (keels) laterally spread (⌘)	-0.23	-0.5
Prey handling	One or more arms inwardly curled while manipulating prey toward beak (⌘)	-0.28	-0.19
Stiff arm tips, keeled	Arms tightly bundled, third arm keels laterally spread (⌘)	0.74	0.02
Arm strike	All arms splayed and suckers extended laterally while attempting to grasp prey ("Strike, arms only," ⌘)	*	*
Strike glide	Stiff arm tips, fins dorsally curled ("Fin curl," †; ⌘)	-0.36	-0.31
Tentacle strike	Arm pairs I, II, and IV splayed; pair III guides extended tentacles toward prey during capture attempt (⌘)	*	*
Trailing tentacles, keeled	Arms bundled and slightly loose anteriorly, third arm keels laterally spread, tentacles held extended beyond arm tips (⌘)	-0.13	0.59
Chromatic		*PC1 (18.3%, 2.95)*	*PC2 (12.4%, 2)*
Bilaterally pale and dark	Body half pale and half dark, laterally divided (⌘)	0.12	-0.12
Countershaded	Body darkest on dorsal surface, shading fades dorsoventrally to pale on ventral surface (⌘)	-0.42	-0.08
Dark	Entire body surface darkened, opposite of "Pale" ("All dark," †; ⌘)	0.16	-0.17
Dark arm stripes	Aboral arm surfaces dark, rest of arms pale (adapted from ⌘)	-0.27	-0.14
Dark arms	Arms all dark (adapted from ⌘)	0.02	0.08
Dark dorsal patch	Dark oviform patch on dorsal mantle surface (⌀)	0.08	0
Dark eye circle	Dark rings encompassing eyes (⌀)	0.15	-0.04
Dark fin edges	Dark outer fin margins terminating at posterior mantle (adapted from ⌘ and "Fin stripe," †)	-0.19	-0.26
Dark fin patch	Darkened patch on dorsal fin surface (⌀)	**	**
Dark fins	Darkened fin dorsal surface (†)	0.02	-0.34
Dark head	Head all dark (adapted from ⌘)	-0.07	-0.06
Dark keels	Darkened dorsal surface of third arm keels (†)	-0.04	-0.09
Dark lateral stripes	Dark horizontal stripe running anteroposteriorly along mantle midline (⌀)	-0.04	-0.08
Dark mantle base	Dark border of anterior mantle edge (⌀)	-0.42	0.24
Inverse countershaded	Body darkest on ventral surface, shading fades to pale on dorsal surface (⌘)	0.07	0.06
Mottle	Irregular, dynamic dark patches covering entire body surface (⌘)	0.15	-0.09
Pale	No chromatophores expanded along entire body surface ("Clear," †; ⌘)	0.23	-0.01
Pale arm tips	Arm tips without pigmentation (adapted from ⌘)	-0.05	-0.01
Pale eyes	Pale rings encompassing eyes (⌀)	0.02	0.08
Pale fin edges	Pale outer fin margins terminating at posterior mantle (⌀)	-0.08	0.46
Pale fins	Fins without pigment (adapted from ⌘)	-0.06	0.1
Pale keels	Pale dorsal surface of third arm keels (⌀)	-0.06	0.22
Pale lateral stripes	Pale horizontal stripe running anteroposteriorly along mantle midline (⌘)	-0.36	-0.15
Pale tentacles	Tentacles without pigment (⌀)	-0.24	-0.2
Pale ventral shield	Pale, iridescent ventral posterior mantle (adapted from ⌘)	-0.2	-0.3
Pale ventral stripe	Pale stripe running anteroposteriorly along ventral mantle surface (adapted from ⌘)	0.08	0.05
Sandy	Entire body uniform grainy coloration of mixed pale and dark (⌘)	0.15	0.28
Shaded eye	Dark band between eyes (†)	0.33	-0.38
Locomotor		*PC1 (52.5%, 1.63)*	*PC2 (19.6%, 0.61)*
Back glide	Slow tail-first locomotion from siphon jet pulses with fins extended laterally (⌘)	-0.41	0.71
Back jet	Fast tail-first locomotion from siphon jetting with fins wrapped around mantle (⌘)	-0.45	-0.69
Back swim	Tail-first locomotion from fin-flapping, sometimes with jetting (⌘)	0.43	-0.12
Forward glide	Slow arms-first locomotion from siphon jet pulses with fins extended laterally (⌘)	0.52	0.1
Forward swim	Arms-first locomotion from fin-flapping, sometimes with jetting (⌘)	0.42	-0.04
† Bell (2011); ⌘ Trueblood et al. (2015); ⌀ present study; *used to define foraging squid and thus excluded from analysis; **performed by fewer that 4 squid and thus excluded from analysis			

Table S1.

Behavioral components recorded and analyzed in *Dosidicus gigas* (n=30), with descriptions and summary statistics from principal component analyses (PCAs) (related to Figure1, Figure 2, Figure S1, and Figure S2). Eigenvalues and variance terms of principal component one (PC1) and two (PC2) are included in parentheses in the component type headings. Postural, chromatic, and locomotor components are defined with the reference(s) for the original description indicated in the bottom row. Eigenvector scores from PCAs are listed for each component.

		ANOVA results					Tukey HSD results				
Ecological category	**Behavior**	*Level*	*DF*	*MS*	*F-value*	*p-value*	*Comparison*	*Difference*	*Lower 95% CI*	*Upper 95% CI*	*adjusted p-value*
foraging status	flashing	foraging status	1	0.016	0.17	0.68	not foraging-foraging	0.046	-0.18	0.27	0.68
		residuals	28	0.093							
	flickering	foraging status	1	1.51	1.38	0.25	not foraging-foraging	0.445	-0.33	1.23	0.25
		residuals	28	1.01							
conspcific abundance	flashing	conspecific abundance	2	0.13	1.44	0.25	few-many	-0.22	-0.55	0.11	0.24
		residuals	27	0.088			some-many	-0.15	-0.48	0.18	0.5
							some-few	0.069	-0.26	0.4	0.86
	flickering	conspecific abundance	2	3.92	4.33	**0.023**	few-many	-1.24	-2.3	-0.19	**0.019**
		residuals	27	0.91			some-many	-0.47	-1.53	0.58	0.51
							some-few	0.77	-0.29	1.82	0.19

Table S2.

ANOVA and Tukey HSD results from a comparison of variable chromatic behavior (*flashing* and *flickering*) duration between factors within ecological categories in *Dosidicus gigas* (see Figure S2B). Significant factors affecting duration, or significant differences between means within a comparison, are shaded in bold (n=30).

Photophore type	Division	Region	Average (±1SD) photophores cm-1	DF	t-value	p-value
small	*whole body*	*whole body*	*4.17 (±0.81)*			
	fins	centers	3.75 (±2.1)	39	-0.12	0.91
	fins	fin edges	9.78 (±4.2)	39	1.59	0.12
	mantle	base	0	39	-1.18	0.24
	mantle	dorsal	2.26 (±0.51)	39	-0.54	0.59
	mantle	lateral stripes	5.07 (±2.1)	39	0.25	0.8
	mantle	ventral	0	39	-1.18	0.24
	mantle	ventral shield	0	39	-1.18	0.24
	mantle	ventral stripe	0	39	-1.18	0.24
	arms and head	arms	12.54 (±4.36)	39	2.37	0.022
	arms and head	dorsal head	35.11 (±13.41)	39	8.77	<0.0001
	arms and head	keels	17.92 (±10.17)	39	3.9	0.0004
	arms and head	tentacles	9.58 (±2.17)	39	1.53	0.13
	arms and head	ventral head	13.11 (±4.38)	39	2.53	0.015
large	*whole body*	*whole body*	*1.44 (±0.3)*			
	fins	centers	0	39	-2.4	0.021
	fins	fin edges	0	39	-2.4	0.021
	mantle	base	7.90 (±1.31)	39	10.76	<0.0001
	mantle	dorsal	0	39	-2.4	0.021
	mantle	lateral stripes	0	39	-2.4	0.021
	mantle	ventral shield	3.05 (±0.85)	39	2.68	0.011
	mantle	ventral	3.24 (±1.03)	39	3	0.0047
	mantle	ventral stripe	4.45 (±2.69)	39	5.013	<0.0001
	arms and head	arms	0	39	-2.4	0.021
	arms and head	dorsal head	0	39	-2.4	0.021
	arms and head	keels	0	39	-2.4	0.021
	arms and head	tentacles	0	39	-2.4	0.021
	arms and head	ventral head	0	39	-2.4	0.021

Table S3.

Regression statistics from linear mixed effects analyses relating the density of small or large photophores to different pigmentation changing regions of *Dosidicus gigas* (related to Figure 3). Each region was compared to the overall whole-body density of each photophore type (which is included in shaded grey boxes for reference). Thus there were two regressions comparing measurements of four squid at each body region. Regions highlighted in yellow indicate those where photophores were significantly more abundant per unit body area than the average whole body density (n=4).

Movie S1 (separate file).

A group of *Dosidicus gigas* changing pigmentation patterning while feeding on lanternfish (Myctophidae) at depth (related to Figure 1, 2). This footage was captured on December 1, 2009 by the Monterey Bay Aquarium Research Institute (MBARI) ROV *Ventana* at 480 m depth in Monterey Canyon, CA (36.70319, -122.04322). Footage © MBARI.

Dataset S1 (separate file).

The datasets generated and analyzed in this study.

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