

Uncovering the function of an enigmatic display: antipredator behaviour in the iconic Australian frillneck lizard

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When faced with a predator, some animals engage in a deimatic display to startle the predator momentarily, resulting in a pause or retreat, thereby increasing their chance of escape. Frillneck lizards (*Chlamydosaurus kingii*) are characterised by a large, pronounced frill that extends from the base of the head to beyond the neck and, when displayed, can be up to six times the width of the head. We used behavioural assays with a model avian predator to demonstrate that their display conforms to deimatic display theory. First, juveniles and adults deployed the frill in encounters with a model predator. Second, the display revealed three colour patches (white and red–orange patches on the frill; yellow mouth palate) that facilitate a transition from a cryptic to a conspicuous state as perceived by a raptor visual system. Third, the display was performed with movements that amplified its effect. The frill area was larger in males than in females, which suggests that the frill might also be co-opted for male–male contests. If future research confirms a role of the frill in male agonistic interactions, frillneck lizards will be a rare case in which a structure has a dual function in a deimatic display and a sexually selected signal.

ADDITIONAL KEYWORDS: *Chlamydosaurus kingii* – conspicuous display – crypsis – deimatic display – reptile – sensory ecology – squamate – startle display – visual display – visual modelling.

INTRODUCTION

The mechanisms involved in antipredator strategies are diverse; animals use crypsis and masquerade to circumvent detection or recognition, aposematism and mimicry to broadcast unpalatability, and motion dazzle and flicker fusion to obscure movement trajectories (Stevens & Merilaita, 2009; Umeton *et al.*, 2017; Loeffler-Henry *et al.*, 2018). These adaptations are all tailored for perception by the visual system of a predator. The leaf-like masquerade of a phasmid, the myrmecomorphy in appearance and locomotion of a jumping spider and the conspicuous wing pattern of a monarch butterfly all function in

portraying a certain morphological state to predators (MacDougall & Dawkins, 1998; Wedmann, 2010; Nelson, 2012). Furthermore, some animals display different phenotypes depending on the identity of the observer. For example, dwarf chameleons exhibit different colour changes for camouflage in response to bird and snake predators (Stuart-Fox *et al.*, 2006, 2008). However, most studies have focused on static signals, such as aposematism, whereby signals are broadcast continuously (Mappes *et al.*, 2005; Stevens & Ruxton, 2012; but see Rowe & Halpin, 2013), whereas signalling coupled with a behavioural response yields a multifaceted strategy during a confrontation with a predator. In the late stages of a predatory threat, some animals will employ a marked transition from an inconspicuous to a highly conspicuous state, called a deimatic display (Maldonado, 1970). This unprecedented transition aims to elicit a ‘startle’

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response in a predator, resulting in a pause or retreat, thereby increasing the performer's chance of escape (Umbers *et al.*, 2015; Umbers & Mappes, 2016).

Deimatic displays are likely to be more common than we think, but they are often overlooked in comparison to other antipredator behaviour and colouration, such as aposematism and camouflage (Umbers *et al.*, 2017). In contrast to warning signals, deimatic displays are dynamic, because they involve a rapid transition from a cryptic to a conspicuous state and do not require predator learning or innate aversion (Umbers *et al.*, 2017; Holmes *et al.*, 2018). Therefore, species that use deimatic displays appear cryptic when in the normal active or resting state because they may, for example, show strong background matching. This behavioural transition from a resting to a display state can be dramatic, and the effect may be amplified by behaviour (e.g. inflation of the body, hissing, gaping of the mouth). The end result is an intimidating display aimed to induce a reflexive response in the receiver (predator).

Frillneck lizards (*Chlamydosaurus kingii* Gray, 1825) are unique among squamates because they have a large, extensible frill that substantially increases the apparent size of the lizards when erect, with the exception of small head flaps in the toad-headed agama (*Phrynocephalus mystaceus* Pallas, 1776; MJW, unpublished observations). Frillneck lizards have long been known to extend their frills outwards (Saville-Kent, 1896); however, despite the novelty of the frill, its function has remained enigmatic. Early hypotheses of the behavioural context of the frill included gliding, food storage, auditory enhancement, crypsis and thermoregulation, none of which have received any empirical or observational support (De Vis, 1883; Fenner, 1933; Bacchus, 1939; Worrell, 1963; Frith & Frith, 1987; Shine, 1990). Only two hypotheses of the function of the frill may be of significance: its use in antipredator behaviour and as a social signal (Shine, 1990). Current behavioural data are insufficient to test the hypothesis of the role of the frill as an antipredator mechanism. Previous data describe a lack of sexual dimorphism in the frill (Shine, 1990), which casts doubt on its role in intrasexual selection.

Here, we hypothesised that the defensive display of the Australian frillneck lizard (*Chlamydosaurus kingii*) conforms to the predictions of deimatic display theory. We predicted that: (1) the frill is erected in response to the presentation of a model predator; (2) exposure of colour patches on the frill creates a rapid transition from a cryptic to a conspicuous state according to the visual system of a predator; and (3) the display is performed in conjunction with behaviours that amplify its effect. We also measured the size of the frill and conspicuousness through the visual system of a lizard to examine differences in sexual dimorphism that might suggest a role in signalling to conspecifics.

MATERIALS AND METHODS

STUDY SYSTEM

The frillneck lizard is a large, diurnal agamid [mean snout–vent length (SVL) 25.4 cm in males and 20.7 cm in females; Shine, 1990] that is locally abundant in savannah woodland habitats across northern Australia, extending from West Australia to southern Queensland and including southern Papua New Guinea (Shine, 1990; Griffiths & Christian, 1996; Cogger, 2002). They are arboreal sit-and-wait predators that prefer habitats with dense canopy cover, a low density of shrubs, and grassy vegetation (Griffiths & Christian, 1996). The most notable feature of frillneck lizards is the extensible frill, in our data spanning up to six times the width of the head and supported by hyoid cartilage.

Fieldwork was conducted from October 2017 to March 2018 at Fogg Dam Conservation Reserve (hereafter, Fogg Dam; 12°35'40.2"S, 131°16'25.1"E) in tropical northern Australia. All methods used were conducted under Northern Territory research permit no. 61517 and approved by Macquarie University Animal Ethics Committee (reference no. 2017/046). Fogg Dam is located along the Adelaide River floodplain and experiences a distinct seasonal wet–dry climate. The study ran from the early to late wet season, with total monthly rainfall varying from 90 to 743 mm and mean monthly daytime temperatures ranging from 28.8 to 32.3 °C (Bureau of Meteorology, 2018). Frillneck lizards show higher activity and growth rates during the wet season, when insect prey is plentiful and lizards are reproductively active (Christian & Bedford, 1995; Ujvari *et al.*, 2015).

Lizards were captured by hand or by noose at night while they slept vertically on eucalypt trunks. For each individual, we measured head height (the distance between the parietal eye and the lower jaw), head width (the distance between the two sides of the lower jaw at the widest part of the head) and head length (the distance between the quadrate–articular jaw joint on the right side to the tip of the snout) to the nearest 0.01 mm using digital callipers, in addition to SVL and tail length to the nearest 1 mm using a standard ruler. We calculated frill area by taking 25 measurements of the left half of the frill, summing the area of the 12 triangular areas, and doubling the result (Supporting Information, Fig. S1). Lizards were sexed by head dimensions (see Supporting Information, Supplementary methods), and any lizard with an SVL < 180 mm was considered to be a juvenile (corresponding to ~1 year of age; Christian *et al.*, 1999). Capture locations were recorded on a GPS (Garmin GPSMAP 64s), and all lizards were released at their point of capture within 24 h.

BEHAVIOURAL ASSAYS AND SCORING

We conducted behavioural assays on 52 lizards (14 males, 22 females and 16 juveniles) at Fogg Dam. Individuals were placed in a cubic arena with wall lengths of 1.5 m. The arena was positioned under a large mimosoid tree with pockets of sunlight, providing sufficient illumination while ensuring that the lizards did not overheat. The sides of the arena were opaque and the top uncovered, meaning that the lizard only had a view of the canopy. All assays were conducted in sunny conditions from 09.00 to 13.00 h. We constructed a model raptor by printing and laminating a digital image of the ventral surface of a peregrine falcon (*Falco peregrinus* Tunstall, 1771) in flight and attaching the centre of the cut-out to a 3 m extendable pole. When shaken, the body of the raptor (29 cm long) remained in position with the stick, while the wings (71 cm wingspan) flapped back and forth with $\sim 80^\circ$ of movement per wing. Down-welling light eliminated glare on the underside of the model and illuminated its outline and pattern.

Each lizard was presented with two treatments separated by 2–3 h, in a random order that was balanced: a model raptor as described above, and a control consisting of a pole without the raptor model attached. Before each assay, the lizard was placed on the ground of the arena in a cloth bag, the bag was lifted, and the lizard was given an acclimation period of 15 min. Either the raptor model or the pole was then placed 0.5 m above the arena (2 m above the ground), and the pole was repetitively moved up and down at a rate of about two flaps per second. The assay occurred for 30 s, and each lizard was subsequently placed back in the cloth bag after this period. Two cameras (Sony FDR-X3000) were mounted on opposite top corners of the enclosure and live-streamed to a handheld iPhone 5s through the PlayMemories Mobile application. This allowed us to orient the model raptor or the pole directly above the lizard for the duration of the assay. The lizard and experimenter were not within view of one another.

From the footage, we scored whether the frill was deployed (details below) in the control and experimental treatments. In the bird model trials, we also scored the number of lunges (L), the number of times the lizard ran/fled from the model (R), and the duration (in seconds) that the lizard had its frill fully erect (F), frill partly erect (P), mouth agape (M) and remained stationary (S). Other associated behaviours included hissing and tail whipping, but these behaviours were difficult to dissociate from other body movements and noises; therefore, they were not quantified in the behavioural scoring. All video footage was scored in BORIS (Friard & Gamba 2016), as was the matrix of transitions after each behaviour. We combined all

behaviours scored to summarize the display behaviour of each individual lizard using eqn:

$$\text{Behavioural score} = \left[\frac{(1/2)(F + M) + (P + 2L)}{30} \right] - \left[\frac{S + 2R}{30} \right]$$

Mouth agape and frill fully erect are only performed in concert with one another; therefore, we halved the summed duration of the two. We considered variables F , M , P and L to be defensive behaviours, whereas S and R were more associated with a ‘flight’ or non-defensive antipredator response. Lizards with a more vigorous display would have a greater display score than lizards with a fleeing or unreactive response. To assess our summary of display behaviour, we also summarized the behaviours, excluding flee, using a principal components analysis (PCA), and the indices agreed strongly (see [Supporting Information, Supplementary methods](#)).

FRILLNECK DISPLAY COLOURATION

‘Colour’ is an interaction of the light environment in the habitat, the spectral reflectance of the animal and the visual system of the receiver (Kemp *et al.*, 2015; Endler & Mappes, 2017), the last of which can only be approximated without behavioural verification. Herein, we use the term for convenience.

We used an Oceanoptics Jaz reflectance spectrophotometer with an illumination probe connected to a PX-2 light source to take spectral reflectance measurements of eight body regions of 52 frillneck lizards. Spectra were standardised by taking measurements at an angle of 90° and 5 mm from the surface, covering an area of 6 mm². Measurements were relative to a dark and a 99% white (WS-1) reflectance standard (Labsphere, Inc.). All raw spectra were obtained from wavelengths 300–700 nm, which encompasses the visual system of lizards and birds (Fleishman *et al.*, 2011; Lind *et al.*, 2013) and were averaged over 5 nm intervals using a kernel smoothing function. We used the application OceanView to retrieve the spectral data, which was processed in R v.3.3.1 using the package PAVO (Maia *et al.*, 2013).

We measured the spectral reflectance of the following regions: dorsum (two locations); edge of the frill (three locations); and the interior of the front of the frill (three locations; [Supporting Information, Fig. S2](#)). For all areas, three measurements were averaged from the same colour patch. The regions along the edge of the frill are visible when the frill is folded (i.e. when the lizard is not in a display state) and are adjacent to the interior colour patches when the frill is extended outwards. The interior of the frill is characterised by a red–orange patch on the lower part of the frill and a white circular patch on the upper part of the frill. Studies have examined similar spectrophotometric

measures of frillneck lizards (Merkling *et al.*, 2016; McLean *et al.*, 2019), although, to the best of our knowledge, palate spectra have not been measured previously. The opening of the frill and mouth are behaviourally coupled, and neither was observed in isolation; therefore, the palate is a potentially important component of the frillneck display.

To quantify the conspicuousness of colour patches, we modelled spectra through both avian and lizard visual perspectives (see ‘Colour analysis’ section below). For each lizard, we used an average of spectra from multiple body regions to represent the resting and display states. The colour of a lizard in a resting state was quantified using two regions along the dorsum and three along the edge of the frill. As arboreal agamids, frillneck lizards occupy vertical eucalypt trunks when in a resting state (Griffiths & Christian, 1996; Supporting Information, Fig. S2), and avian predators are most likely to spot them from perches. When examined from an avian perspective, from above, the white patches are positioned laterally and are not visible; therefore, we did not include the white patches in the resting state (Supporting Information, Fig. S2). The measures of conspicuousness for the resting state were taken as the average of two regions on the dorsum and three regions on the edge of the frill against the average reflectance of 30 samples of eucalypt bark. Bark samples were collected from the study site at perches from which the lizards had previously been captured.

The colour of a lizard when in the display state was quantified using the red–orange patch, the white patch and the palate, which were analysed separately (Supporting Information, Fig. S2). The red–orange patch is almost entirely confined to the bottom three frill folds and is obscured when the lizard is at rest. Two elliptical white patches are positioned directly to the sides of the open mouth and exposed palate. Measures of conspicuousness for the display state were taken as the three interior colour patches against the average of the three adjacent regions on the edge of the frill.

VISUAL MODELLING

We modelled frillneck lizard colouration through the visual system of a bird predator. Whistling kites (*Haliastur sphenurus* Vieillot, 1818) and black kites (*Milvus migrans* Boddaert, 1783) are abundant birds of prey at Fogg Dam (Sergo & Shine, 2015) and are likely to be the main predators of frillneck lizards. Given that microspectrophotometry data are unavailable for these species, we chose the common buzzard (*Buteo buteo* Linnaeus, 1758) as a close relative in the Accipitriformes to model raptor vision.

To explore the hypothesis that the frill plays a role in male–male signalling whereby male frills will be more conspicuous than in females, we also modelled colouration as perceived by a lizard conspecific. We used photoreceptor sensitivity data from the ornate crevice-dragon (*Ctenophorus ornatus* Gray, 1845), which is also an agamid, because lizard visual systems are conserved (Fleishman *et al.*, 2011) and no data exist currently for the frillneck lizard.

To estimate the ability of an avian predator or lizard conspecific to discriminate between different colour patches, we applied Vorobyev–Osorio receptor-noise models (Vorobyev & Osorio, 1998). The model uses signal intensity (photoreceptor quantum catches) and receptor noise to estimate distances in perceptual space between spectra in units of ‘just-noticeable differences’ (JNDs; see Supporting Information, Supplementary methods for model specifications and details).

STATISTICAL ANALYSES

All statistical analyses were performed in R v.3.3.1 (R Core Team 2018). Before all analyses, we explored the data following the protocol of Zuur *et al.* (2010) for validity of test assumptions and integrity of the data. During this process, missing values were removed from the data set. Most notably, palate spectra were not recorded for one individual, and this explains the discrepancy in sample size and degrees of freedom between the models (see Tables 1 and 2). Also before analyses, we used a rank-transformation to normalise our behavioural display score (Riley *et al.*, 2017).

First, we wanted to examine whether the lizards (males, females and juveniles combined) erected the frill in response to a predator. To accomplish this, we used Pearson’s χ^2 test (*chisq.test* function in the *stats* R package; R Core Team, 2018) with Yates’ continuity correction (Stefanescu *et al.*, 2005) to compare presence/absence of frill erection in 51 lizards between the experimental and control assays.

Second, we examined differences between males, females and juveniles in conspicuousness (in JNDs) of the white patch and red patch of the frill, the palate and the dorsum for raptor (*B. buteo*) and lizard (*C. ornatus*) visual systems, in addition to frill area (in square centimetres) and display behavioural scores. For each of these response variables, we ran linear models, using the function *lm* in the R package *stats* (R Core Team, 2018), that included the continuous variable of SVL and the categorical variable of sex [three levels: female, male and juvenile (indeterminate sex)]. Models were run initially with an interaction effect between SVL and sex, but if this effect was not significant the models were re-run without the interaction effect to allow accurate interpretation of the main effects. If

Table 1. Least-squares means and pairwise comparisons of chromatic and achromatic contrasts of colour patches according to the visual system of the common buzzard (*Buteo buteo*), representing an avian predator

Sex	Least-squares means				Pairwise differences of contrast				P-value			
	Mean	SE	d.f.	95% Confidence interval	Contrast	β	SE	d.f.		t		
											Lower bound	Upper bound
Chromatic contrast												
White patch	Female	4.260	0.575	47	3.103	5.416	Female-juvenile	-7.674	1.707	47	4.495	< 0.001
	Juvenile	11.934	1.519	47	8.877	14.990	Female-male	4.369	1.497	47	2.918	0.005
	Male	-0.109	1.475	47	3.076	2.857	Juvenile-male	12.043	2.826	47	4.261	< 0.001
Red patch	Female	7.095	0.786	47	5.514	8.676	Female-juvenile	-1.011	2.333	47	-0.433	0.667
	Juvenile	8.106	2.076	47	3.929	12.283	Female-male	4.127	2.047	47	2.017	0.050
	Male	2.968	2.015	47	-1.087	7.023	Juvenile-male	5.138	3.863	47	1.330	0.190
Palate	Female	5.806	0.548	46	4.702	6.910	Female-juvenile	-5.085	1.710	46	-2.974	0.005
	Juvenile	10.891	1.570	46	7.731	14.051	Female-male	4.021	1.541	46	2.609	0.012
	Male	1.785	1.491	46	-1.216	4.787	Juvenile-male	9.106	2.904	46	3.135	0.003
Dorsum	Female	2.416	0.440	47	1.531	3.301	Female-juvenile	-6.942	1.306	47	-5.315	< 0.001
	Juvenile	9.359	1.162	47	7.020	11.697	Female-male	4.680	1.146	47	4.085	< 0.001
	Male	-2.264	1.128	47	-4.534	0.006	Juvenile-male	11.623	2.163	47	5.374	< 0.001
Achromatic contrast												
White patch	Female	11.958	0.556	47	10.839	13.078	Female-juvenile	-6.512	1.652	47	-3.942	< 0.001
	Juvenile	18.470	1.470	47	15.513	21.427	Female-male	3.893	1.449	47	2.687	0.010
	Male	8.066	1.427	47	5.195	10.936	Juvenile-male	10.405	2.735	47	3.805	< 0.001
Red patch	Female	6.363	0.581	47	5.193	7.532	Female-juvenile	-5.760	1.726	47	-3.337	0.002
	Juvenile	12.123	1.536	47	9.033	15.213	Female-male	2.910	1.514	47	1.922	0.061
	Male	3.453	1.491	47	0.453	6.452	Juvenile-male	8.670	2.858	47	3.034	0.004
Palate	Female	12.075	0.655	46	10.756	13.394	Female-juvenile	-4.198	2.044	46	-2.054	0.046
	Juvenile	16.273	1.876	46	12.496	20.050	Female-male	2.151	1.842	46	1.168	0.249
	Male	9.924	1.782	46	6.337	13.511	Juvenile-male	6.349	3.471	46	1.829	0.074
Dorsum	Female	4.131	0.617	47	2.891	5.372	Female-juvenile	-3.105	1.830	47	-1.696	0.097
	Juvenile	7.236	1.629	47	3.959	10.513	Female-male	2.194	1.606	47	1.367	0.178
	Male	1.937	1.581	47	-1.244	5.118	Juvenile-male	5.299	3.030	47	1.749	0.087

Bold P-values indicate significance at a level of $\alpha = 0.05$.

Table 2. Least-squares means and pairwise comparisons of chromatic and achromatic contrasts of colour patches according to the visual system of the ornate crevice-dragon (*Ctenophorus ornatus*), representing a frillneck lizard conspecific

		Least-squares means					Pairwise differences of contrast					
Sex	Contrast	Mean	SE	d.f.	95% Confidence interval	Contrast	β	SE	d.f.	<i>t</i>	<i>P</i> -value	
												Lower bound
Chromatic contrast												
White patch	Female	4.883	0.853	47	3.167	6.599	Female–juvenile	-7.638	2.532	47	-3.016	0.004
	Juvenile	12.520	2.253	47	7.987	17.054	Female–male	4.443	2.221	47	2.000	0.051
	Male	0.440	2.187	47	-3.961	4.840	Juvenile–male	12.081	4.192	47	2.882	0.006
Red patch	Female	6.220	0.816	47	4.578	7.863	Female–juvenile	-1.841	2.424	47	-0.760	0.451
	Juvenile	8.061	2.157	47	3.722	12.401	Female–male	3.948	2.126	47	1.857	0.070
	Male	2.272	2.094	47	-1.940	6.484	Juvenile–male	5.789	4.013	47	1.443	0.156
Palate	Female	8.500	0.853	46	6.782	10.217	Female–juvenile	-5.947	2.661	46	-2.235	0.030
	Juvenile	14.447	2.443	46	9.529	19.365	Female–male	5.242	2.398	46	2.186	0.034
	Male	3.257	2.320	46	-1.413	7.928	Juvenile–male	11.190	4.519	46	2.476	0.017
Dorsum	Female	2.963	0.898	47	1.156	4.770	Female–juvenile	-8.288	2.667	47	-3.108	0.003
	Juvenile	11.251	2.373	47	6.477	16.025	Female–male	4.221	2.339	47	1.804	0.078
	Male	-1.258	2.304	47	-5.892	3.377	Juvenile–male	12.509	4.415	47	2.833	0.007
Achromatic contrast												
White patch	Female	17.863	0.808	47	16.238	19.489	Female–juvenile	-6.098	2.399	47	-2.542	0.014
	Juvenile	23.961	2.135	47	19.667	28.256	Female–male	4.141	2.104	47	1.968	0.055
	Male	13.722	2.072	47	9.553	17.891	Juvenile–male	10.239	3.972	47	2.578	0.013
Red patch	Female	11.127	0.851	47	9.416	12.838	Female–juvenile	-5.337	2.525	47	-2.114	0.040
	Juvenile	16.464	2.247	47	11.943	20.985	Female–male	3.313	2.215	47	1.496	0.141
	Male	7.814	2.181	47	3.425	12.202	Juvenile–male	8.651	4.181	47	2.069	0.044
Palate	Female	17.907	1.079	46	15.736	20.078	Female–juvenile	-3.124	3.364	46	-0.929	0.358
	Juvenile	21.030	3.088	46	14.814	27.247	Female–male	1.041	3.032	46	0.343	0.733
	Male	16.866	2.933	46	10.962	22.770	Juvenile–male	4.165	5.713	46	0.729	0.470
Dorsum	Female	4.874	0.843	47	3.178	6.570	Female–juvenile	-8.696	2.503	47	-3.475	0.001
	Juvenile	13.570	2.227	47	9.090	18.050	Female–male	6.167	2.195	47	2.809	0.007
	Male	-1.293	2.162	47	-5.642	3.056	Juvenile–male	14.863	4.143	47	3.587	< 0.001

Bold *P*-values indicate significance at a level of $\alpha = 0.05$.

a main effect of sex was found to be significant, we used a post hoc test of least-squares means (using the *lsmeans* R package with the functions *lsmeans* for main effect comparisons and *lstrends* for interaction effect comparisons; [Lenth, 2016](#)) to examine pairwise differences of all contrasts between the sexes. For all models, before interpretation, we verified the assumptions of normality and homoscedasticity of residuals. All linear models were set to a significance level of $\alpha = 0.05$.

RESULTS

FRILL DISPLAY AND BEHAVIOUR

Lizards erected their frills in response to the model avian predator more often than in response to the control pole ($\chi^2 = 34.16$, d.f. = 1, $P < 0.001$). Of the 52 lizards, 43 (83%) of them remained unresponsive to the control pole. The other eight lizards (17%) erected their frills with their mouths agape in response to the control pole. In response to the avian model, 38 lizards (73.1%) erected the frill with their mouth agape, three lizards (5.8%) fled, and the remaining 11 lizards (21.2%) remained unresponsive. All lizards that erected the frill initially did so < 0.5 s after presentation of the model. Of the 38 lizards that deployed the frill initially, 50% lunged, 13.2% fled and 18.4% stopped displaying (i.e. remained stationary). Of the 19 lizards that lunged, 63.2% subsequently fled. No behaviours were considered after 'flee' in the behavioural sequence because the boundary of the arena might have affected their natural behaviour ([Fig. 1](#)).

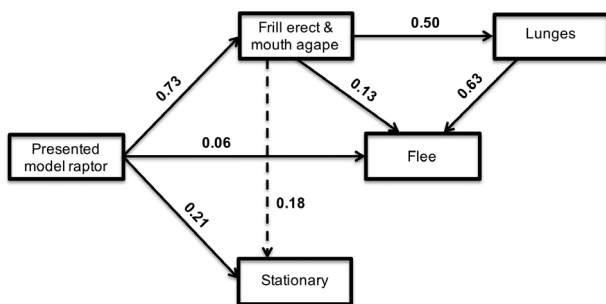


Figure 1. The antipredator behavioural sequence of 52 frillneck lizards in response to the presentation of a model raptor predator, showing the frequencies of transitions after each behaviour. Behaviours are inside the boxes, the proportions are adjacent to the arrows, and the dashed line denotes a non-overlapping arrow. Flee (*F*) and stationary (*S*) were considered endpoints of the sequence. Other associated behaviours included tail lashes and hissing, although these were not easily discernible in the behavioural assays.

COLOURATION FROM THE PERSPECTIVE OF A RAPTOR
The red patch, white patch and palate of lizards ([Fig. 2](#)) were all distinguishable from their respective contrast regions from the visual perspective of a raptor; JNDs were greater than a discrimination threshold value of one ([Fig. 3](#)). In addition, the brown dorsum was distinguishable against the tree background.

Just-noticeable differences of chromatic contrast were higher for females than males in the white patch, red patch, palate and dorsum. Juvenile JNDs of chromatic contrasts were higher than for females and males in the white patch, palate and dorsum ([Table 1](#); [Fig. 4](#)). Just-noticeable differences of achromatic contrast were higher in females than in males in only the white patch. Juvenile JNDs of achromatic contrast were higher than for females in the white patch, red patch and palate, and higher than for males in both the white patch and the red patch ([Table 1](#)). All other colour patches were not different between sexes. Chromatic and achromatic contrast in the white patch and the red patch, in addition to dorsum chromatic contrast, were significantly related to SVL ([Supporting Information, Table S1](#)). No other colour patches were related to SVL ([Supporting Information, Table S1](#)).

COLOURATION FROM THE PERSPECTIVE OF A LIZARD
Through an agamid visual system, JNDs were higher for females than for males in palate chromatic contrast and dorsum achromatic contrast ([Table 2](#)). Juveniles were greater than both males and females in JNDs of chromatic contrast in the white patch, palate and dorsum, and in achromatic contrast in the white patch and the red patch ([Table 2](#)). All other colour patches were not different between sexes. Achromatic contrast in the white patch, red patch and dorsum were significantly related to SVL, but this relationship did not exist for any other colour patches ([Supporting Information, Table S2](#)).

SEXUAL DIMORPHISM OF THE DISPLAY OF THE FRILLNECK LIZARD

We found a significant interaction effect between SVL and sex for frill area ($\beta = 18.566$, SE = 4.994, $t = 3.717$, $P = 0.006$). Male frill area was the largest and increased more steeply with SVL (greater slope) than both females ($\beta = -26.508$, SE = 13.061, d.f. = 42, $t = -2.029$, $P = 0.049$) and juveniles ($\beta = -26.060$, SE = 12.345, d.f. = 42, $t = -2.111$, $P = 0.041$; [Fig. 5](#)). Females had larger frills than juveniles; however, the relationship between SVL and frill area was not significantly different ($\beta = -0.448$, SE = 5.629, $t = -0.080$, $P = 0.937$; [Fig. 5](#)).

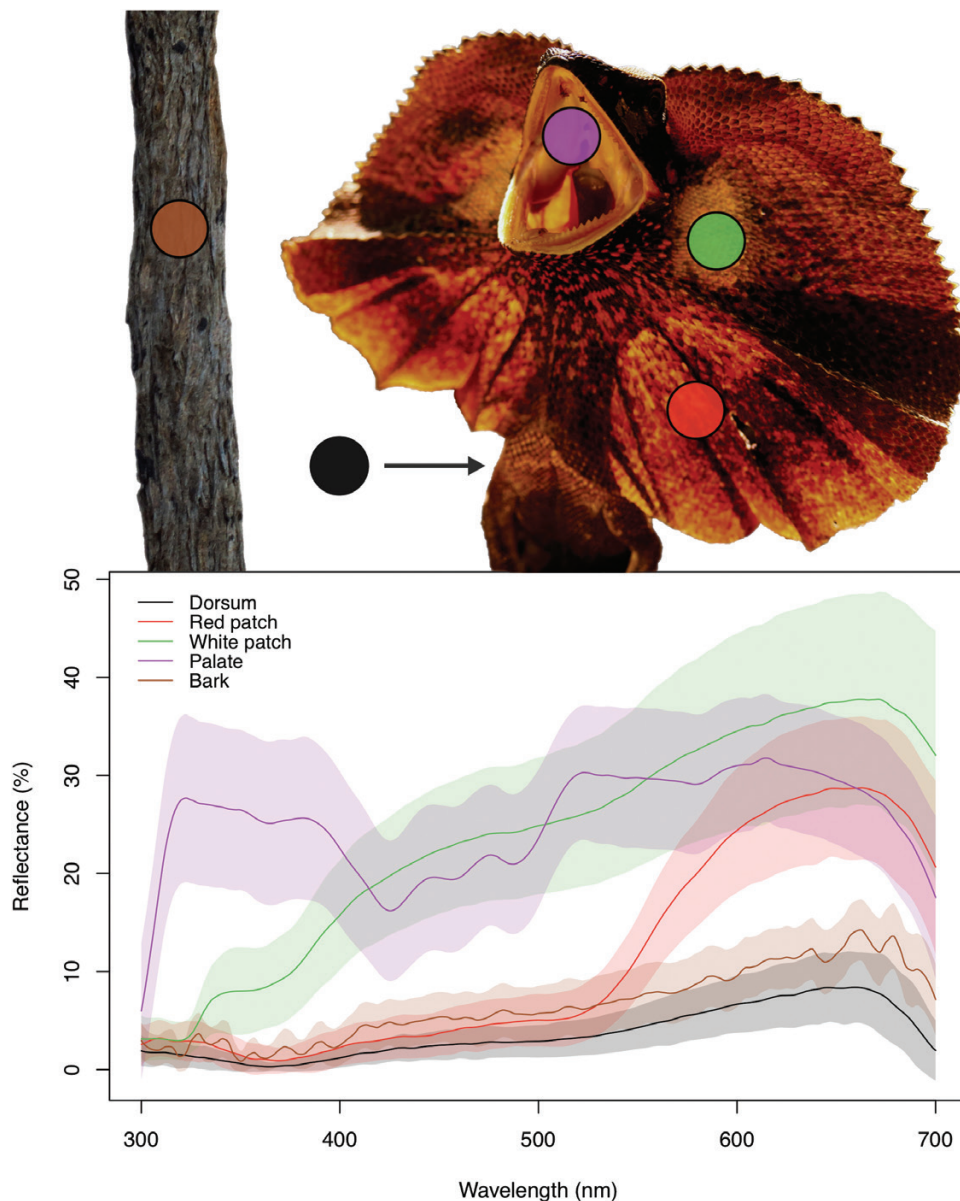


Figure 2. Mean spectra \pm SE of four colour patches of 52 frillneck lizards and one of the bark background: dorsum (black line and shading), represented by three regions on the lizard's body and three regions on the edge of the frill; red–orange patch (red line and shading); white patch (green line and shading); palate (purple line and shading); and eucalypt bark (brown line and shading).

We also found differences between sexes in display behaviour. Males had a higher display score than both females ($\beta = -0.691$, SE = 0.295, d.f. = 48, $t = -2.342$, $P = 0.023$) and juveniles ($\beta = -0.676$, SE = 0.321, d.f. = 48, $t = -2.106$, $P = 0.04$), but we found no significant difference between females and juveniles ($\beta = -0.016$, SE = 0.289, d.f. = 48, $t = -0.054$, $P = 0.958$).

DISCUSSION

In accordance with our hypothesis, frillneck lizard antipredator behaviour was consistent with deimatic display theory. Frillneck lizards erected their frills in response to the model avian predator significantly more often than to the control pole. In response to a predatory threat in controlled conditions, lizards

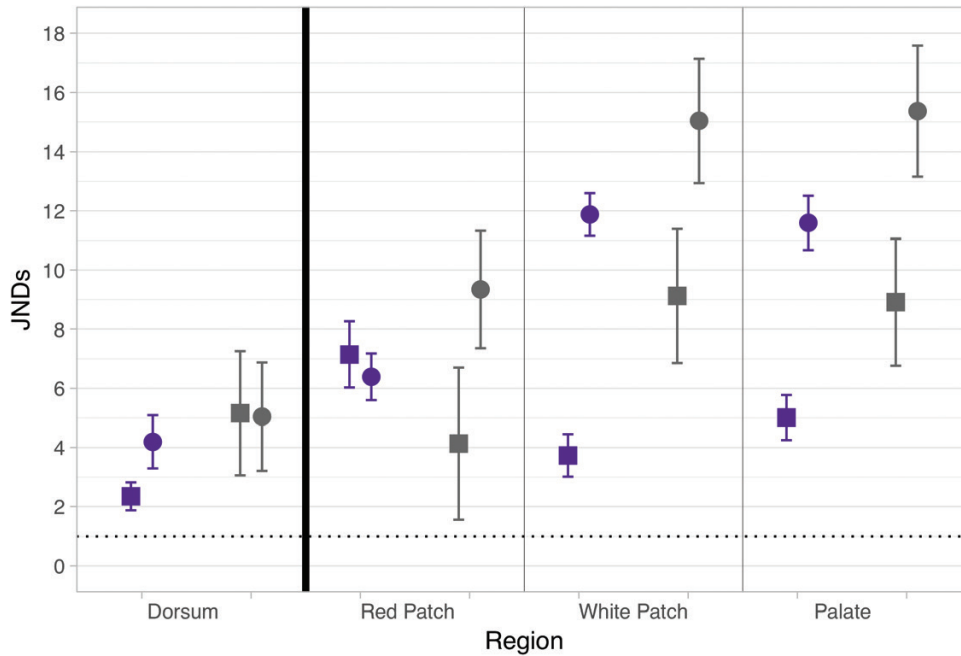


Figure 3. Means \pm 95% confidence intervals of chromatic (square) and achromatic (circle) contrasts of four colour patches against the dorsum of the lizard in adults (purple) and juveniles (grey) in units of just-noticeable differences (JNDs) using a raptor visual system. The vertical bold line demarcates the resting (left) and display (right) states, and the horizontal dotted line indicates a discrimination threshold value of one for the JNDs (Siddiqi *et al.*, 2004).

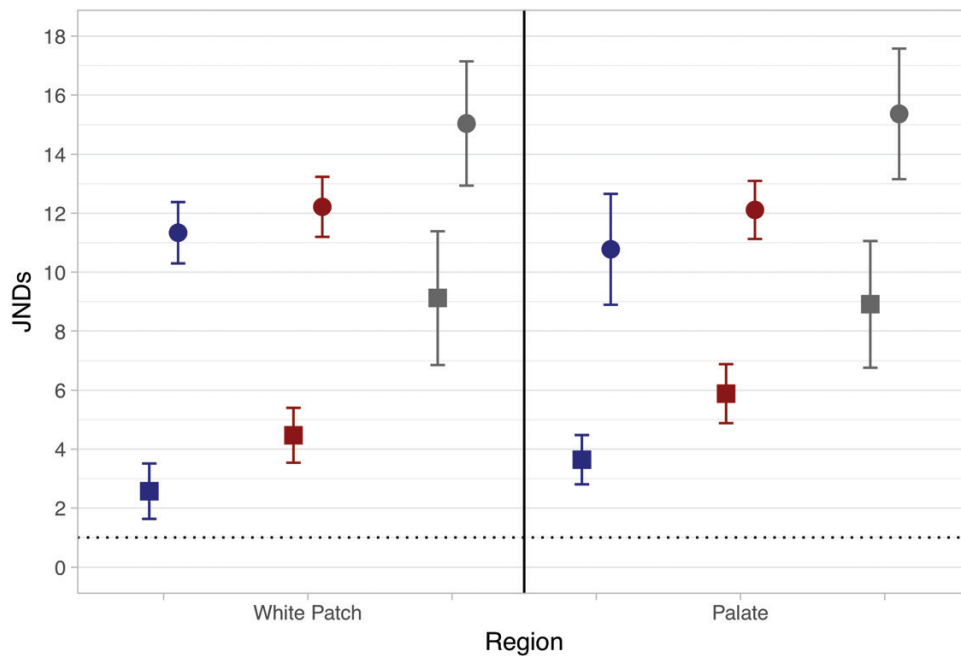


Figure 4. Means \pm 95% confidence intervals of chromatic (square) and achromatic (circle) contrasts of the white patch and palate against the dorsum of the lizard in males (blue), females (red) and juveniles (grey) in units of just-noticeable differences (JNDs) using a raptor visual system. The horizontal dotted line indicates a discrimination threshold value of one for the JNDs (Siddiqi *et al.*, 2004).

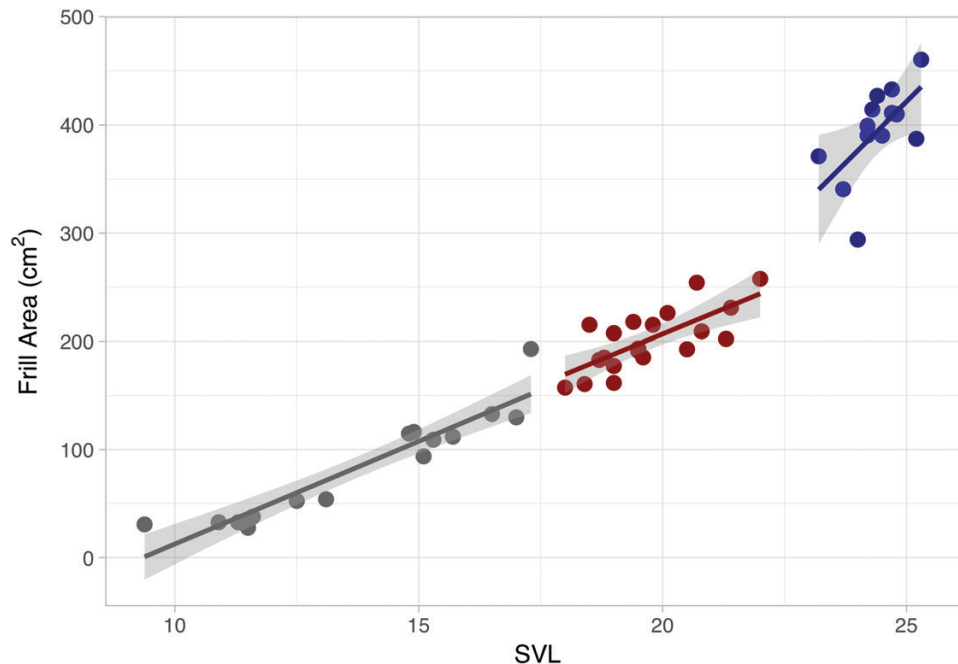


Figure 5. Frill area in relationship to snout–vent length (SVL) across males (blue), females (red) and juveniles (grey), with superimposed fitted lines over data points and shaded 95% confidence intervals.

exhibited a marked transition from a cryptic, resting state to an active, display state. During rest, the lizard remained stationary, with concealed colour patches. In the display state, lizards erected the frill in concert with colour patches on the frill and palate, which are highly conspicuous to a raptor visual system. Frill erection was accompanied by behaviours (lunging, swaying, hissing and tail-whipping) oriented at the predatory threat. Warning signals can often involve multiple components and different sensory modalities (Rowe & Guilford, 1999), thereby maximizing the effect. Likewise, in a deimatic display, the abrupt presentation of a visual stimulus may be concurrent with movements, sounds or chemicals that amplify its effect (Vallin *et al.*, 2005; Umbers & Mappes, 2015; Umbers *et al.*, 2017).

Erecting the frill considerably increases the apparent size of the lizard from the perspective of a predator; the structure spans up to six times the width of the head in adult males. Due to frill size alone, the misjudgement of prey size may stall or halt the predatory sequence. Moreover, the frill itself may serve as a diversion from the body; it is fragile and susceptible to tears and damage; therefore, attacks concentrated on the frill would be less likely to incapacitate or gravely injure the lizard.

Frill displays were triggered by the introduction of the model raptor into the visual field of the lizard. In all frillneck lizards that performed the display, the behaviour was initiated simultaneously with the

appearance of the model avian predator over the arena (within 0.20 s from the moment the model bird was presented). Lizards are likely to deploy the frill during the final stages of a predatory encounter. A late-stage encounter is likely to have a greater impact on the sensory system of a predator by virtue of proximity. Behavioural observations by Shine (1990) and field observations by C.A.P.-M. support the hypothesis that lizards primarily initiate their displays at close range. However, frillneck lizards may also display from a distance of 10–50 m; these cases are uncommon and have mainly been noted in response to a vehicle (Shine, 1990). Perhaps, in these cases, the magnitude of the threat triggers the display, because vehicles are large, fast-moving and loud.

In addition to behaviour, colouration of the frillneck lizard transitions from a cryptic to conspicuous state during their display. The brown dorsum of the lizard is distinguishable from the bark background; however, given that the lizard remains motionless during the resting state it is likely to be relatively camouflaged to an avian predator. The erection of the frill exposes patches on the frill and palate, all of which are highly conspicuous to a raptor visual system. The spectral reflectance of the palate revealed a strong ultraviolet (UV) component. In northern blue-tongued skinks (*Tiliqua scinoides intermedia* Mitchell, 1955), the tongue is UV, conspicuous and thought to be a key component of a deimatic display (Badiane *et al.*, 2018). In frillneck lizards, the palate is always exposed

during displays, and we suggest that the UV-reflective mouth acts as an additional component of the display, amplifying the effect. Lastly, the two white patches strongly contrast with the surrounding red, orange and black colouration. Given the large frill as a backdrop, we speculate that the white patches might convey the appearance of eyespots to a predator. Overall, the conspicuousness of the colouration of the frill adds to the effect of the display by the frillneck lizard and increases the potential of the display to elicit a startle response in a predator.

Frillneck lizards are quick and agile. On the contrary, most animals that perform deimatic displays are relatively slow-moving, at least to the extent that they would be unlikely to escape a predator once they are confronted, such as the blue-tongued skink (Badiane *et al.*, 2018). When possible, we expect frillneck lizards to flee and remain hidden from predators as their first line of defense, only erecting the frill when cornered or when escape seems improbable. Our enclosed arena did not allow lizards this possibility, but we found that 17 of the 38 lizards that erected their frills subsequently fled from the model until they made contact with a side of the enclosure. It is possible that if assays were performed in a larger enclosure, we might have observed a higher proportion of lizards that fled after displaying and, possibly, a higher proportion of lizards that fled initially.

The toad-headed agama is another fast-moving agamid whose antipredator behaviour conforms to deimatic display theory (MJW, unpublished observations). This species runs from an approaching predator, and displays its head flaps only when it is 'ambushed' and restrained by a model predator (e.g. the subjugation phase, which is when a predator bites the animal; Vermeij, 1982). We speculate that early in the evolution of the frill, frillneck lizards might have had similar timing of the display, in which a small proto-frill would be most effective as a predator deterrent when a predator comes into contact with the lizard. As larger frills were selected for, the timing of their display behaviour might have shifted as the structure became effective in predator deterrence from a distance. This shift would allow the lizard to retain the ability to startle predators while lowering the probability of physical harm.

Juvenile frillneck lizards showed the greatest conspicuousness of both chromatic and achromatic contrast to both an avian and a lizard receiver. This suggests that survival of smaller, younger and more vulnerable dragons might rely on more conspicuous display colouration. The predators differ between life stages for many reptiles, because of a dramatic change in body size during growth or a change in habitat use (Irschick *et al.*, 2000; Keren-Rotem *et al.*, 2006; Llewelyn *et al.*, 2012; Purwandana *et al.*, 2016). If frill

conspicuousness changes throughout the lifetime of an individual, the question arises as to whether juveniles face a different suite of predators, or predation pressure, compared with adults. For example, because juveniles have colour patches with greater conspicuousness to a lizard receiver than adults, it is possible that juveniles are at a higher risk from predation by lizard predators, including conspecifics and goannas (e.g. *Varanus panoptes* Storr, 1980). Juvenile frillneck lizards also inhabit smaller trees and are more likely to perch in dense understorey (C.A.P.-M., personal observation), which might predispose them to different predation pressure. This may imply that the targeted receiver of the deimatic display by the frillneck lizard might change as the animal matures. In the juvenile life-stage, our findings suggest that the display might target both avian and lizard predators. Future research on deimatic displays should consider how it might change throughout the lifetime of an individual.

Male competition for access to females and/or resources can also drive the evolution of conspicuous traits, resulting in sexual dimorphism (Andersson, 1982; Berglund *et al.*, 1996; Macedonia *et al.*, 2002). Exaggeration of male traits, particularly sexual dichromatism, is common in lizards (Stuart-Fox & Ord, 2004) and can be used as an intraspecific signal to assess fighting ability without escalation into physical combat (e.g. Whiting *et al.*, 2003, 2006, 2015). Frill colour has been proposed as a signal used in territorial displays between males (Shine, 1990; Christian *et al.*, 1995). In our study and others, males have been documented to have larger frills than females (Christian *et al.*, 1995), although Shine (1990) found no evidence of sexual dimorphism in frill size. If the frill is a rare structure with a dual role in deimatism and intrasexual selection, we would expect the frill to have differences in morphology and/or conspicuousness of colour patches to a lizard visual system, with males having larger and more conspicuous frills (e.g. Whiting *et al.*, 2003; Stuart-Fox & Ord, 2004). In line with this hypothesis, our data showed that males had larger frills than females and juveniles, along with a disproportionate increase in frill area in relation to SVL (Supporting Information, Table S2). Males also had a significantly greater display score and displayed their frills more vigorously than females. However, male and female frills did not differ in their colouration according to a lizard visual system, with the exception of dorsum achromatic contrast and palate chromatic contrast (Table 2). Therefore, sexual selection might operate primarily on frill size and display behaviour as opposed to frill colour. Exactly how the frill is potentially used to signal resource-holding potential, male quality or fighting ability is a fruitful avenue for future research.

Deimatic displays are captivating examples of antipredator behaviours, yet much empirical work remains to be done to understand their role in the survival of animals. Our results establish that the use of the frill by *Chlamydosaurus kingii* in antipredator behaviour conforms to the predictions of deimatic display theory. The display is momentary, transient and highly conspicuous, accompanied by behaviours that amplify its effect. To our knowledge, there are exceedingly few documented observations of frill erection in response to a predator *in situ* (an exception is the head flaps in the toad-headed agama). We presume that the highly conspicuous frill elicits a reflexive response in an avian predator (and perhaps other types of predators), although this remains to be tested. Frillneck lizard frill colouration was more conspicuous in juveniles, potentially reflecting a change in the nature of the deimatic display by this species across ontogeny. Supporting this hypothesis, the sexual size dimorphism in the frill and behavioural difference in the display (males having larger frills and more vigorous displays) hints at a possible dual role in antipredator behaviour and sexual selection. If this is true, it will be a rare instance of an antipredator display being co-opted for another function (social signalling). We recommend that future research should examine the behavioural responses of predators to frillneck lizard displays, in addition to staged dyadic contests to test the role of the frill in contest competition.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website:

Supplementary methods. Sexing wild-caught lizards, display score summary and visual modelling.

Figure S1. Diagram of measurements taken to calculate frill area. Yellow lines (2–4) correspond to hyoid cartilaginous segments. White lines (5–25) are used to represent the remainder of half of the frill.

Figure S2. Photographic representation of the resting and display states of the frillneck lizard, with corresponding regions where we took spectrophotometric measurements.

Figure S3. Graphical representation of principal components analysis (PCA) for head morphology of male (blue), female (red) and juvenile (grey) frillneck lizards. Circles represent 95% confidence intervals. The PCA included head height (in millimetres), head width (in millimetres) and head length (in millimetres) measurements for 52 lizards. All morphological measurements were \log_{10} -transformed.

Table S1. Output of linear models exploring whether sex and snout–vent length are predictors of chromatic and achromatic contrasts of colour patches according to the visual system of: (1) the common buzzard (*Buteo buteo*), representing an avian predator; and (2) the ornate-crevice dragon (*Ctenophorus ornatus*), representing a frillneck lizard conspecific. Bold *P*-values indicate significance at a level of $\alpha = 0.05$, and reference levels of the categorical variable sex are presented in parentheses after its name.

Table S2. Output of a linear model exploring whether sex or snout–vent length is a predictor of frill area. Bold *P*-values indicate significance at a level of $\alpha = 0.05$, and reference levels of the categorical variable sex are presented in parentheses after its name. Post hoc multiple comparisons between effects can be found in the main text.

Table S3. Component loadings for first (PC1) and second (PC2) principal components from our principal components analysis (PCA) for head morphology of frillneck lizards. The PCA included \log_{10} -transformed head height (in millimetres), head width (in millimetres) and head length (in millimetres) measurements for 52 lizards. We also present the standard deviation of PC1 and PC2, in addition to the proportion of variance they explain in our data. See the [Supporting Information \(Fig. S3\)](#) for a graphical representation of these data.

Table S4. Component loadings for the first principal component (PC1) from our principal components analysis (PCA) for display behavioural scores. The PCA included the behavioural categories used in the formula for behavioural score in the main text in the main text, with the exception of flee (*R*) for 52 lizards. We also present the standard deviation of PC1 and the proportion of variance it explains in our data.

SHARED DATA

All data and R code from this study can be accessed at doi:10.17605/OSF.IO/98F23.

Supporting Information for

Uncovering the function of an enigmatic display: antipredator behaviour in the iconic Australian frillneck lizard

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29 October 2019

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Supplementary Methods

Sexing wild-caught lizards

Lizards were sexed by head dimensions because adult frillneck lizards show sexual dimorphism in head shape (Christian et al. 1995). A principal component analysis (PCA) was performed in R version 3.3.1 (R Development Core Team 2014) using the function *PCA* from the R package *FactoMineR* (Husson et al. 2014). The PCA was used to summarize measurements of head height (mm), head width (mm), and head length (mm). The first principal component (PC1; relative size) explained 98.39% of the variation, and PC2 (longer and taller head shape) explained 1.11% of the variation (see Table S3 for PC loadings). We plotted PCs with 95% confidence intervals using the *ordiplot* function in the *vegan* R package (Oksanen et al. 2017). Males and females formed distinct clusters in morphospace (Fig. S5), and a snout-vent length cut-off value of 18 cm was used to separate adults from juveniles. Previous studies used 16.8 cm and 17.5 cm as cut-offs for sexually mature females, though we are being conservative with our designation of adult frillneck lizards (Griffiths and Christian 1996, Uvjari et al. 2015).

Display score summary

Using the same protocol as above, we ran a PCA using the number of lunges (*L*), and the duration that the lizard had its frill fully erect (*F*) and partially erect (*P*), mouth agape (*M*), and remained stationary (*S*). The number of times the lizard fled (*F*) was excluded from the PCA, because in preliminary analysis we found that the direction this variable loaded did not reflect its biological intention. We used PC1 as a display score, which explained 59.9% of the variation in the data (see Table S4 for PC loadings). As PC1 increased it reflected a less vigorous display — lizards made fewer lunges, had a lower proportion of time with the frill fully erect, partially erect and mouth agape, and a greater proportion of time stationary. We assessed if this display score (PC1) reflected our calculated display score (see Formula 1 in the manuscript) using a Pearson's product-moment correlation (*cor.test* function in the *stats* R package; R Core Team 2018). We found that both display scores were highly correlated ($R^2 = 0.933$, $t = -75.985$, $df = 50$, $P < 0.001$). Due to the similarity between both display scores, we use the calculated display scores in statistical analyses.

Visual modelling

We measured irradiance using an irradiance channel on an Oceanoptics Jaz spectrometer and took measurements at 90° to the ground on sunny, clear days between 10:00-13:00 h across three days in February 2018. We used the same 30 perches from which bark samples were taken and took three measurements per perch, corresponding to different directions in horizontal space. We averaged 90 measurements to produce an irradiance curve and converted it to flux for the visual modelling.

Photoreceptor sensitivity is calculated as the product of the visual pigment spectral sensitivity, the oil droplet transmittance, and the transmittance of the ocular media (Lind et al., 2013). We followed the methods of Hart and Vorobyev (2005) by using the SWS1 pigment sensitivity of 405 nm (Ödeen & Håstad, 2003) for *B. buteo* to estimate the transmittance spectra of T-, C-, Y-, and R-type oil droplets. We then used the peak spectral sensitivities: 407 nm (violet-sensitive; VS), 471 nm (short-wavelength sensitive; SWS), 538 nm (medium-wavelength sensitive; MWS), and 602 nm (long-wavelength-sensitive; LWS) and ocular media transmittance data from Lind et al. (2013) to reproduce the visual system of the common buzzard. It has been postulated that the double cone mediates luminance perception in birds (Osorio et al., 1999; Goldsmith & Butler, 2005, Lind & Kelber, 2011). However, recent findings provide evidence that many raptors including the common buzzard lack double cones in the central and temporal foveae, suggesting that single cones likely contribute to achromatic vision in birds (Mitkus et al., 2017). Antibodies for violet and green cones were detected in the common buzzard foveae and suggest that all single cone types are likely present, given the argument that an animal loses the violet cone first (A. Kelber, pers comm). Therefore, we used the sum of all cone types to model avian achromatic discrimination.

We applied the Vorobyev-Osorio receptor-noise model to estimate an avian predator's ability to discriminate between different colour patches (Vorobyev & Osorio, 1998). The model uses signal intensity (photoreceptor quantum catches) and receptor noise to estimate distances in perceptual space between spectra in units of "just-noticeable differences (JNDs)." We assumed the photoreceptor noise (u.) for the LWS photoreceptor to be 0.1 for chromatic contrast based on *Leiothrix lutea*, and 0.15 for achromatic contrast based on *Aquila audax* (Reymond & Wolfe, 1981; Maier, 1992). A ratio of 1:2:2:4 was used for the relative photoreceptor densities (Vorobyev & Osorio, 1998), and we applied the Von Kries transformation for photoreceptor adaptation to the light environment (Fleishman et al., 2011).

We used similar methodology for modelling a lizard visual system to examine how a frillneck lizard perceives a conspecific. Although Barbour et al. (2002) did not identify ultraviolet-sensitive (UVS) photoreceptors in *C. ornatus*, studies have confirmed the presence of UVS pigments in *C. decresii* and other related lizards (Loew et al., 2002; Bowmaker et al., 2005; Yewers et al., 2015). Yewers et al. (2015)

suggest that the VS/UVS cone is difficult to detect through microspectrophotometry and is likely present in *Ctenophorus* spp., so a UVS cone was considered in the visual modelling (*sensu* Chan et al., 2009; Merklings et al., 2016). Wavelengths of maximum absorbance 365 nm (UVS), 440 nm (SWS), 493 nm (MWS), and 571 nm (LWS) and the A1-based visual pigment were used to yield the alpha-band and beta-band, then summed to produce the pigment sensitivity functions (Govardovskii et al., 2000; Barbour et al., 2002). We multiplied the curves by ocular media transmittance with a cut-off at 350 nm (Bowmaker et al., 2005) and by the oil droplet transmittance to produce photoreceptor sensitivities. Oil droplet cut-off values (λ_{cut}) were estimated to be 425 nm (MWS), and 520 nm (LWS), while UVS and SWS cones were assumed to be transparent (Barbour et al., 2002; Stuart-Fox 2007). For achromatic discrimination we used the *C. ornatus* double cone since this is consistent with visual modelling of other lizards (e.g., Stuart-Fox et al., 2007; Macedonia et al., 2009; Fleishman et al., 2011). We used the receptor-noise model with the following parameters: a Weber fraction of 0.1 for both achromatic and chromatic contrast (Vorobyev & Osorio, 1998; Fleishman et al., 2011), and a ratio of 1:1:3.5:6 for the lizard photoreceptor classes with Von Kries transformation (Fleishman et al., 2011, Maia & White, 2018).