Experience overrides colour in lizard contests

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Summary

We examined the role of conspicuous coloration in male-male contests for two species of Australian dragon lizards, *Ctenophorus decresii* and *C. vadnappa*, in which conspicuous coloration has a demonstrated predation cost. We conducted contests in which the overall conspicuousness of male coloration was manipulated using paints that matched the spectral reflectance of the lizards, as well as natural (control) contests. There was little evidence for an influence of colour on contest outcome or aggression levels for either species when all experiments were considered. However, we found a significant effect of trial order and experience on contest outcome and aggression levels (the same pair of males was used for both types of contest), despite a 2-3 week interval between contests. When we examined only the first trial between unfamiliar males, we found that male *C. vadnappa* that had been painted to appear more conspicuous consistently won. Comparison with the natural trials suggests that the aspect of colour manipulation that was responsible for this result was the 'hue' of the throat: males with yellower throats consistently beat males with bluer throats in both natural and painted trials. The difference in coloration of flank markings also predicted the difference in aggression scores between contestants in the natural trials. These results suggest that although colour is important in opponent assessment and in determining contest outcome in *C. vadnappa*, previous agonistic experience can override the effects of colour and have a long-lasting influence on aggressive behaviour.

Keywords: experience effect, male competition, signalling, sexual selection, Agamidae.

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Introduction

Theory predicts that when individuals place similar value on a contested resource, fighting effort (aggression) and contest outcome should be determined by asymmetries between contestants in qualities such as size, ornamentation and experience (Maynard Smith & Price, 1973; Parker, 1974; Enquist & Leimar, 1983; Taylor & Elwood, 2003). While size is the most commonly reported type of asymmetry that predicts contest success, many other types of asymmetry between individuals may influence contest outcome (Andersson, 1994; Maynard Smith & Harper, 2003). For instance, visual signals such as conspicuous colour patches and other ornaments may signal fighting ability or status in male contests and predict outcome because they facilitate accurate opponent assessment. Accurate assessment of the relative fighting ability of a prospective opponent may enable individuals to avoid potentially costly, escalated contests (Maynard Smith & Harper, 2003). Motivation and prior agonistic experience (wins or losses against familiar and/or unfamiliar opponents) may also be strong predictors of contest success (e.g., Jackson, 1991; Khazraie & Campan, 1999; Hofman & Schildberger, 2001; Maan, 2001; Moretz, 2003; Moretz & Morris, 2003). Indeed, there is growing evidence that factors affecting motivation, such as prior agonistic experiences, can be as important or even more important than asymmetries in phenotypic traits (e.g., size and coloration) in determining contest outcome (Lemel & Wallin, 1993; Zucker & Murray, 1996; Mathis & Britzke, 1999; Daws et al., 2002). To date, however, experience effects appear to be generally short-lived (Chase et al., 1994; Hsu & Wolf, 2001) and their interaction with asymmetries in phenotypic traits in contests, particularly over longer inter-contest intervals, remains poorly understood (Beaugrand, 1997).

Here, we explore the roles of spectral characteristics of conspicuous, sexually dimorphic throat, flank and chest colour patches as well as experience in male contests in two species of agamid lizard, *Ctenophorus decresii* and *C. vadnappa*. *C. decresii* and *C. vadnappa* are two closely related species of rock dwelling agamids endemic to southern Australia. Both species exhibit marked colour variation both within and among populations (Houston, 1998), especially in the conspicuous throat and flank markings of males. Experimental evidence demonstrated that conspicuous coloration in males of these species incurs a cost due to predation and that brighter males suffered higher predation risk (Stuart-Fox et al., 2003, 2004). The predation cost of conspicuous coloration in these species must be offset by a benefit in terms of increased mating opportunities, suggesting that sexual selection may play an important role in the evolution of colour variation in these lizards. Males of both *C. decresii* and *C. vadnappa* also exhibit elaborate aggressive displays which emphasise those aspects of their colour that differ most strongly from females (Gibbons, 1977, 1979; Johnston, 1997) and are presumably polygynous like other rock dragons (Johnston, 1997; Lebas, 2001). Males are strongly territorial (Gibbons, 1979), suggesting that prior agonistic experience with both neighbours and strangers may play an important role in male contests (Stamps & Krishnan, 1998; Whiting, 1999; Lopez & Martin, 2001). The predation cost of conspicuous male coloration and the mating system of rock dragons suggest that both colour and agonistic experience may both be important determinants of contest outcome and ultimately, reproductive success in rock dragons.

We tested whether conspicuous coloration of the flank and throat predicted aggression levels and contest success. To do this, we conducted both 'natural' contests as well as contests in which the overall conspicuousness of male coloration was manipulated using paints that matched the spectral reflectance of the lizards. This latter experiment isolated the role of conspicuous male coloration from other morphological, behavioural or olfactory traits that may influence the outcome of male contests. Previous attempts to isolate the role of colour from behavioural or olfactory cues in male-male contests using experimental manipulations of lizard coloration have not tested whether the paints used resemble the coloration of the lizards spectrally, complicating interpretation of results (e.g., Sigmund, 1983; Losos, 1985; Cooper & Burns, 1987; Thompson & Moore, 1991; Martin & Forsman, 1999; Whiting et al., 2003). We also examined the effect of prior contests on aggression levels and contest outcome and discuss the interaction between asymmetries in colour traits and prior experience in aggressive encounters in these lizards.

Methods

Lizard collection and husbandry

Lizards of both species were collected from the Flinders Ranges in South Australia. A total of 38 male *C. decresii* and 34 male *C. vadnappa* were used in experiments. Lizards were housed individually in outdoor enclosures

(50 × 80 × 80 cm). All lizards were provided with water ad libitum and maintained on a diet of mealworms (*Tenebrio* sp.) dusted with multivitamin and calcium powder, supplemented with crickets, maggots, cockroaches and locust nymphs. Lizards were captured in December 2000 and experiments conducted from February to April 2001. Male colour develops at the onset of sexual maturity and is maintained throughout the year.

Male colour and morphology

Measurements of both snout-vent length (SVL) and mass were taken at the beginning and end of the trial period. The mean of these two measurements was used to represent male traits at the time of the experiments. Condition was calculated as the residuals of weight regressed against SVL (Jakob et al., 1996).

Spectral reflectance was measured using an Ocean Optics S2000 spectrometer and a deuterium-tungsten DT1000 light source, both connected to a bifurcated fibre optic probe. The probe was mounted within a probe-holder that allowed readings to be taken from an oval area 3.5 × 5 mm, at a constant distance of 1 cm from the surface. The end of the tubular probe holder was cut at a 45° angle such that illumination was at 45° relative to the surface, and reflectance measured at the same angle following established protocols (Endler, 1990). Measurements were expressed relative to a 99% diffuse white reflectance standard. Dark current and white standard measurements were taken before measuring each lizard. Lizards were placed in a tank with a basking area under a heat lamp for at least 15 minutes prior measurement. Reflectance was measured after lizards had attained their preferred body-temperature (35-40°C; Gibbons, 1977), determined using a thermo-couple. Measurements were taken for each of three different body regions: chest, throat, and orange flank markings (Figure 1). These body regions were chosen because they differ in colour between males and females (Houston, 1974) and contrast most with the natural rock background in males (Stuart-Fox et al., 2003, 2004). Measurements were then averaged over 5 nm intervals between 320-700 nm to facilitate data manipulation. Measurements were taken at three different locations for each body region and the mean used as the representative spectrum. Variation in spectral reflectance was summarised using Principal Components Analysis (PCA) (Cuthill et al., 1999). A separate PCA was conducted on reflectance spectra for each of the three body regions (Cuthill et al., 1999).

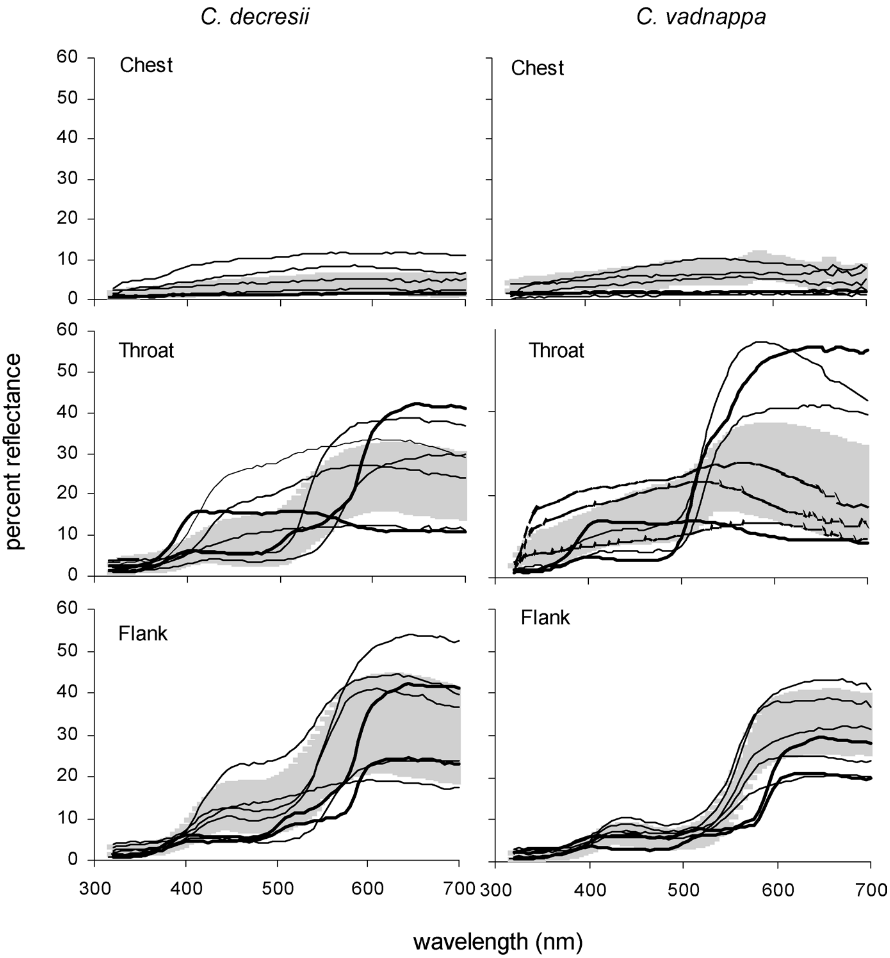

Figure 1. Reflectance spectra of lizard colours showing individual variation. For simplicity, 5 males were chosen to represent the range of variation in spectral shape (thin black lines). Areas shaded grey represent one standard deviation on either side of the mean for all males used in trials for each species. Thick black lines represent the spectral reflectance of paints used for colour manipulation. Both contestants were given the same size and colour chest patch. 'Bright' males were given throat and flank colours that correspond to the thick black line with higher percent reflectance. Conversely, 'dull' males were given throat and flank colours that correspond to the thick black line with lower percent reflectance.

Male contests

Two types of encounters were staged, natural and painted. In the natural encounters, we compared contest outcome and aggression levels for size matched conspecific males. Males were ranked and paired to minimise size and mass differences because SVL and mass may affect the outcome of male contests and may mask effects of coloration. The maximum difference between contestants was 6.8% SVL and 10.5% mass (SVL: mean = 3.0±2.0% SD, mass: mean = 4.3 ± 3.1% SD) for any pair of *C. decresii* and 3.9% SVL and 9.1% mass (SVL: mean = 1.8 ± 1.1% SD, mass: mean = 4.4 ± 2.9% SD) for any pair of *C. vadnappa*. Effects of coloration in natural trials may also be confounded with other factors that are more difficult to control for, such as other morphological, behavioural or olfactory traits. Therefore, to isolate the role of colour, we also conducted two trials in which colour was manipulated using paints, a common procedure in experimental studies on lizards (e.g., Sigmund, 1983; Losos, 1985; Cooper & Burns, 1987; Thompson & Moore, 1991; Martin & Forsman, 1999; Whiting et al., 2003). In one trial, contestant A was painted to appear more conspicuous ('bright') and contestant B to appear less conspicuous ('dull') and in the second trial, the treatments were reversed so that contestant A appeared 'dull' and contestant B appeared 'bright' (see below for details of colour manipulation). In these painted encounters, the same size matched pair of males was used so that all traits except coloration were 'held constant', thereby allowing us to isolate the effects of colour from other morphological, behavioural or olfactory cues that may influence contest outcome.

Overall, each lizard pair was used three times, once in a natural trial and twice in trials in which contestant colours were manipulated using paints. All trial types, both natural and painted, were performed over the same three-month period with trials involving the same males being separated by a two to three week period. Between trials, each male was housed in its own enclosure and was not in visual contact with other males. The order of all three trials for each pair of lizards (one natural trial and two painted trials) was randomised. The long inter-trial period and randomisation of trial order were both designed to control for possible effects of time, experience and motivation. However, we nevertheless tested for an effect of contest order on contest outcome and aggression levels.

Contests were conducted in an arena 40 × 40 cm with a raised tile in the middle. Lizards were allowed to bask beneath heat lamps for 20 minutes before the trial and the trial arena was placed on an electric blanket set at 35°C so that body temperatures could be maintained close to preferred temperatures during the trials (36°C, Gibbons, 1977). Trials were videotaped and lasted 35 minutes as pilot experiments, conducted one year prior, had shown 30 minutes to be sufficient time to establish a clear winner and loser in 7 out of 7 cases. Minor injuries (skin abrasions) occurred in two of a total of 108 trials conducted and there were no apparent signs of stress during or after the trial period.

Contest outcome (winner/loser) was easily determined because the loser either repeatedly attempted to escape or crouched in a corner of the enclosure. Male aggression was measured as a weighted index of various aggressive behaviours. There were five classes of aggressive or dominant behaviour: (1) biting; (2) hind-leg pushup display (slow pushup on all four limbs incorporating tail coiling, lowering of gular region and dorso-lateral flattening — see Gibbons, 1979); (3) chase; (4) aggressive posturing (lowering of gular region, dorso-lateral flattening) and (5) tail flick. The first two behaviours were given a weight of 3, the second two were given a weight of 2 and the last was weighted 1. This weighting scheme was based on previous detailed behavioural studies that documented male aggressive displays and their order of escalation in both *C. decresii* and *C. vadnappa* (Gibbons, 1977, 1979). The weights are a conservative index of relative aggression reflected by each behaviour and the overall aggression score is analagous to those used in a wide variety of experimental studies of male contests (e.g., Losos, 1985; O'Connor et al., 1999; Whiting, 1999; Hagelin, 2002). Aggression scores were log-transformed to meet assumptions of normality for use of parametric statistics.

Manipulation of male colour

In the manipulated contests, both males had their colours manipulated using non-toxic Chromacryl® acrylic paint to produce more conspicuous or less conspicuous coloration. Males vary most in throat and flank colour, rather than chest patch colour or brightness (Figure 1). For this reason, we controlled for variation in chest patch size and colour by painting chest patches the same colour and size on both contestants. The more conspicuous treatment involved painting males to have bright orange flank markings and throat for *C. decresii*, and bright orange flank markings and yellow throat for *C. vadnappa*. In the less conspicuous treatment, males were painted to have dull orange flank markings and grey-blue throats in both species. These treatments were the same as those used in experimental tests showing a predation cost of 'bright' coloration relative to 'dull' coloration (Stuart-Fox et al., 2003). Reflectance spectra of paints used in both types of treatment were matched as closely as possible to reflectance spectra of the most and least conspicuous males we found in the field (Figure 1, see Stuart-Fox et al., 2003 for details).

Statistical analysis

Morphological variation

To determine whether colour traits were dependent on male size or condition, we tested for a correlation between these variables and principal components (PCs) describing the spectral reflectance of each body region, using Pearson correlations with sequential Bonferroni adjustments for multiple tests (Rice, 1989).

Effect of contest order

First, we tested whether the same male consistently won contests. We used a binomial test with an expected probability of 0.25 that a male who won the first fight would win both subsequent fights, where the outcome of these two fights is independent of the first. To test whether trial order affected aggression levels, we compared aggression levels of males in their first, second and third trials using ANOVA with the fixed factor 'contest' identifying whether it was the first, second or third contest for each individual and 'lizard pair' as a random factor identifying each pair of lizards. Accordingly, the factor 'contest' was tested against contest × lizard pair as the error term.

Natural trials

For the natural male contests, we first examined whether any traits were associated with winning or losing. We conducted a multiple logistic regression of win/loss against the following predictor variables: PC1 and PC2 for the flank and throat; PC1 of the chest; SVL and condition. For this analysis, we randomly chose a winner or loser from each contest as the behaviour of winners and losers within each contest are interdependent. We used stepwise model selection with the significance cut-offs for both a variable's initial inclusion and exclusion from the model set to $p = 0.1$.

To determine whether aggression (rather than outcome) was associated with any of the colour or other morphological variables, we tested for associations between the difference in aggression score between contestants and respective differences in the predictor variables, again using multiple regression. The difference in all variables was calculated as the value for the winner minus the value for the loser. The predictor variables were the same as those used in all previous analyses and the multiple regressions were conducted using the same selection procedure and criteria as above.

Painted trials

First, we tested whether painting had an effect on overall aggression levels by comparing the aggression level of males in the unpainted trials with their mean aggression level in the two painted trials, again using ANOVA. Second, the two reciprocal experiments, where male colour was manipulated, were analysed using ANOVA with a 2 × 2 Latin Square design, which takes proper account of the inter-dependence between contestants and between trials (Petersen, 1985). This analysis tested for an effect of treatment (being painted 'bright' or 'dull') on aggression levels.

Results

Colour variation

The first two principal components (PCs) in most analyses accounted for over 80% of the variation in the variables involved (Table 1). Therefore only the first two PCs were used in statistical analyses, with one exception. We used only the first PC for chest colour because it accounted for at least 90% of variation in both species. This is because there was very little variation in chest spectral shape, the chests of both species being essentially black or shades of grey.

The biological interpretation of the principal components (PCs) was determined by examining the correlations of principal component eigenvectors with wavelength (5 nm intervals; Cuthill et al., 1999). For both species, PC1 for the colours of the three body regions, flank, throat and chest, correlated with mean reflectance and therefore represents brightness, while PC2 reflects variation in spectral shape (Cuthill et al., 1999). The exception is the throat of *C. vadnappa*, where PC1 indicates variation in short relative to medium-long wavelengths and differentiates between males with yellow throats and males with grey or yellow-grey throats. PC1 of the throat of *C. vadnappa* can therefore be viewed as analogous to 'chroma' or 'saturation'. PC2 of the throat

Table 1. Percent variation and cumulative variation accounted for by each by the first two principal components (PCs) from separate principal components analyses (PCAs) on the colour of different body regions. Biological interpretation of each PC is also given — see text for explanation.

Traits	PC	*C. decresii*			*C. vadnappa*		
		% Variation	Cumulative % variation	Interpretation	% Variation	Cumulative % variation	Interpretation
Throat	PC1	52	52	‘brightness’	47	47	‘saturation’
	PC2	31	83	‘hue’	41	88	‘hue’
Flank	PC1	67	67	‘brightness’	60	60	‘brightness’
	PC2	16	83	‘hue’	28	88	‘saturation’
Chest	PC1	90	90	‘brightness’	99.9	99.9	‘brightness’
	PC2	6	96	Not included	0.1	100	Not included

Table 2. Correlations of size (SVL) and condition with colour variables for *C. decresii* and *C. vadnappa.* Values that are significant at $p < 0.05$ after Bonferroni correction for multiple tests are denoted by an asterisk.

Species	Colour variable	Correlation with SVL		Correlation with condition	
		r	*p*	*r*	*p*
C. decresii	Chest PC1	0.069	0.68	0.072	0.67
	Throat PC1	0.062	0.71	0.019	0.91
	Throat PC2	0.254	0.12	−0.204	0.22
	Flank PC1	−0.421	0.008*	−0.065	0.7
	Flank PC2	−0.024	0.89	0.248	0.133
C. vadnappa	Chest PC1	−0.046	0.81	−0.208	0.27
	Throat PC1	−0.05	0.79	0.277	0.14
	Throat PC2	0.299	0.11	−0.132	0.49
	Flank PC1	−0.012	0.95	0.442	0.016
	Flank PC2	0.225	0.24	0.251	0.19

differentiates between yellow and blue throated males and can therefore be viewed as corresponding to ‘hue’. In *C. vadnappa*, PC2 of the flank indicates variation in medium-long relative to shorter wavelengths. Males with higher PC2 had more saturated flank markings. In *C. decresii*, PC2 of the throat differentiates males with yellow or orange throats from males with blue-grey throats. Similarly, PC2 of the flank in *C. decresii* differentiates males with orange markings from males with blue-grey markings so in both these cases, PC2 corresponds to ‘hue’.

Correlations between colour, size and condition

Of the five colour variables, only the brightness of the orange flank markings was negatively correlated with size (SVL) in *C. decresii* (Table 2). None of the colour variables was correlated with condition in *C. decresii*. Similarly, in *C. vadnappa* none of the colour variables were correlated with size or condition after Bonferroni correction for multiple tests (Table 2).

Male contests

Natural trials

None of the independent variables (colour variables, size, condition) predicted the probability of winning or losing for either species (Table 3). Sim-

Table 3. Multiple regressions testing for associations contest outcome/aggression and predictor variables. A backwards stepwise procedure was used for all models. $N = 19$ for *C. decresii* and $N = 15$ for *C. vadnappa*. NA in all columns denotes that no variable explained a significant portion of the variation the model.

Test	Species	Variable	Coefficient	S.E.	r^2	F or Wald χ^2	p
Multiple logistic regression of win/loss against predictor variables	*C. decresii*	–	–	–	–	–	NA
	C. vadnappa	–	–	–	–	–	NA
Multiple regression of difference in aggression scores against difference in predictor variables	*C. decresii*	–	–	–	–	–	NA
	C. vadnappa	Intercept	1.38	0.19		52.26	<0.0001
		PC2 flank	0.07	0.03	0.30	5.18	0.042

Table 4. ANOVAs for male contest trials with colour manipulated. ‘Treatment’ identifies whether the lizard was painted ‘bright’ or ‘dull’; ‘Lizard pair’ identifies each pair of lizards; ‘Trial’ identifies the two contests fought by each pair of lizards and is therefore nested within lizard pair. The factor ‘treatment’ was tested against Treatment × Lizard pair as the error term.

Species	Factor	Sum of squares	df	*F*	*p*
C. decresii	Treatment	0.04	1	0.17	0.68
	Lizard pair	14.24	18	0.77	0.72
	Trial (Lizard pair)	6.26	19	0.32	0.99
	Treatment × Lizard pair	3.65	18	0.2	0.99
	Error	19.50	19		
C. vadnappa	Treatment	0.27	1	1.05	0.31
	Lizard pair	6.61	14	0.40	0.96
	Trial (Lizard pair)	4.27	15	0.24	0.99
	Treatment × Lizard pair	3.44	14	0.21	0.99
	Error	17.65	15		

ilarly, the degree of differences in colour, size or condition between contestants did not predict the difference in their aggression levels for *C. decresii* (Table 3). However, in *C. vadnappa*, the difference in flank ‘chroma’ between contestants predicted their difference in aggression score (Table 3). This indicates that when the individual with more saturated orange flank markings won (positive difference in flank PC2), the contest was characterised by large differences in aggression. Conversely, when the individual with less saturated orange flank markings won (negative difference in flank PC2), the contest was characterised by small difference in aggression. Examination of the raw data indicated that small differences in aggression generally indicated low aggression displayed by both contestants.

Painted trials

There were no significant differences in aggression levels when individuals were unpainted and when they were painted (*C. decresii*: $F_{1,18} = 0.19$, $p = 0.66$; *C. vadnappa*: $F_{1,14} = 1.07$, $p = 0.31$) indicating that painting did not affect natural behaviour. However, manipulating colour had no effect on male contest success for either species when all trials were considered (Table 4). In neither species was there an overall effect of treatment (being painted ‘bright’ or ‘dull’) on aggression scores (Table 4). Although sample sizes are small for both natural and painted trials ($N = 19$ for *C. decresii*;

$N = 15$ for *C. vadnappa*), several other similar studies on the role of colour in contests (in birds and lizards) have detected large effect sizes (>0.5), often with smaller sample sizes ($N = 9$-14, e.g., Thompson & Moore, 1991; Olsson, 1994a; references in McGraw & Hill, 2000). Thus, although we lack power to detect subtle effects, we can conclude that *C. decressii* and *C. vadnappa* do not exhibit the strong effects of coloration on contest success evident in several other taxa.

Effects of contest order

For both species, males who won the first encounter won both the subsequent two encounters significantly more often than expected by chance (*C. decresii*: 13/18, Binomial test: $z = 4.63$, $p < 0.001$; *C. vadnappa*: 11/14, Binomial test: $z = 4.63$, $p < 0.001$).

There was also a significant effect of trial order on aggression levels, despite the two to three week interval between contests (*C. decresii*: $F_{2,38} = 5.34$, $p = 0.008$; *C. vadnappa*: $F_{2,32} = 3.92$, $p = 0.03$). In *C. decresii*, aggression levels were significantly higher in the first trial than in the second two trials, in which aggression levels were similar (contest 1 vs 2: $t_{35} = 3.0$, $p = 0.005$; contest 1 vs 3: $t_{35} = 2.67$, $p = 0.01$; contest 2 vs 3: $t_{35} = -0.45$, $p = 0.65$). In *C. vadnappa*, aggression in the first trial was significantly higher than in the second trial ($t_{29} = 3.33$, $p = 0.002$), but not significantly higher than in the third trial ($t_{29} = 1.39$, $p = 0.18$). Aggression scores in the second and third trials were not significantly different ($t_{29} = -1.15$, $p = 0.25$).

Initial contests between unfamiliar males

Because we found a significant effect of previous fighting experience, we subsequently examined only the first contest between pairs. Out of the initial contests between unfamiliar males, approximately one third were natural and two thirds were painted as the order of natural and painted trials had been randomised. In *C. decresii*, males that had been painted ‘bright’ won 7 of 13 manipulated contests (Binomial test: $z = 0.28$, $p = 0.78$), although power analyses ($\alpha = 0.05$, power $= 0.80$) indicate that with a sample size of 13, only differences as large as 10 out of 13 contests could be detected. In *C. vadnappa*, males that had been painted bright won 8 out of 8 manipulated contests (Binomial test: $z = 2.83$, $p = 0.005$). Thus, male *C. vadnappa* that were painted to appear more conspicuous won significantly more of their initial trials than expected by chance despite the small sample size, indicating a very strong effect.

In order to isolate the aspect of colour manipulation that contributed most to this result, we also examined the six natural trials between unfamiliar *C. vadnappa* males. In *C. vadnappa*, the conspicuous paint colours had higher values of both flank PCs and the first PC of the throat, but lower values of PC2 of the throat than the less conspicuous treatment. Of the natural contests, males with higher values for the flank PCs and first PC of the throat won 1 out of 6 trials, whereas 6 out of 6 trials were won by males with lower values of PC2 of the throat (Binomial test: $z = 2.45$, $p = 0.014$). Thus, in 14 out of 14 trials between unfamiliar males (8 painted plus 6 natural; Binomial test: $z = 3.74$, $p < 0.001$), males with yellower throats won over males with bluer throats.

Discussion

Contest outcome was not predicted by colour traits in either *Ctenophorus decresii* or *C. vadnappa* when all contests were considered. Similarly, aggression levels in natural trials were not associated with these traits although in *C. vadnappa*, the difference in flank coloration predicted the difference in aggression between contestants. Despite the 2-3 week interval between contests, we detected a significant effect of prior contests on subsequent contest outcome and aggression levels. In both species, males that had won the first contest won their next two contests more often than expected by chance and males were more aggressive in their first encounter than in their second encounter. These results suggest that previous contest experience may override effects of coloration in determining aggressive behaviour in these lizards. To explore this possibility, we examined only the first contest between unfamiliar males. We found that in *C. vadnappa*, but not *C. decresii*, males painted to be more conspicuous won significantly more trials than expected by chance. The failure to detect a significant treatment effect when considering all trials is therefore likely to be due to an experience effect: males that had won/lost their first contests won/lost subsequent contests despite a different colour treatment. These results suggest that colour does influence aggressive behaviour, at least in *C. vadnappa*, but that previous experience is a more important determinant of contest outcome. Several other studies on a range of taxa including lizards have similarly found a stronger effect of experience than asymmetries in coloration and that experience effects can override effects of asymmetries in size or coloration, which otherwise predict contest outcome (Lemel & Wallin, 1993; Zucker & Murray, 1996; Mathis & Britzke, 1999; Daws et al., 2002).

That colour is important in opponent assessment in *C. vadnappa* was suggested by results of both the natural and painted trials. In all natural and painted trials between unfamiliar opponents, the male with the yellower throat won, although the importance of other aspects of throat and flank coloration cannot be discounted as sample sizes were small and we lacked sufficient power to detect subtle effects of coloration. This is consistent with results for several other lizard species in which colour signals have been shown to affect the outcome of male contests and function as badges of status, reliably indicating male fighting ability (Thompson & Moore, 1991; Olsson, 1994a; Sinervo & Lively, 1996; Whiting et al., 2003). In addition, in the natural trials, contests in which males with more saturated orange markings won were characterised by large differences in aggression while contests in which males with less saturated orange markings won were characterised by small differences in aggression and low overall aggression, despite the effects of previous fighting experience. Although male *C. vadnappa* with more saturated orange flank markings did not consistently win contests, these results suggest that flank coloration may be used in opponent assessment and decisions on whether or not to escalate a contest. Our results for *C. vadnappa* are consistent with the hypothesis that predation costs associated with conspicuous coloration are offset by benefits that may ultimately increase mating opportunities. In a closely related congener, *Ctenophorus ornatus*, 65% of offspring are sired by males other than the one whose territory is shared by the female (Lebas, 2001) and contests between unfamiliar males may be important in obtaining extra-territorial copulations. However the maintenance of territorial boundaries, and hence access to females within the territory, may be influenced to a greater degree by prior experience and/or established relationships with neighbours (McMann, 1993; Stamps & Krishnan, 1998; Whiting, 1999; Lopez & Martin, 2001).

Given that *C. vadnappa* showed a strong relationship between colour and aggression and contest outcome, why was colour unrelated to aggression or contest outcome in *C. decresii* (despite the larger sample size for this species)? One possibility is that colour variation in *C. decresii* is highly complex. While male *C. vadnappa* exhibit only blue and yellow throat colour combinations, male *C. decresii* have throats with varying combinations and patterns of blue, black, yellow and orange. It is therefore possible that the colour combinations that we chose to manipulate coloration in *C. decresii* did not correspond to natural coloration associated with fighting ability, that is, painted individuals may have been broadcasting 'mixed signals' (Marchetti, 1998). In addition, we did not consider the size of colour patches, which may be a more important aspect of colour signals than the spectral qualities of the colour itself. Finally, conspicuous coloration in *C. decresii* may have functions other than signalling fighting ability. Other investigations have demonstrated that conspicuous colouration in lizards may communicate information about species, sex and individual identity (e.g., Sigmund, 1983; Andrews, 1985; Losos, 1985; Hess & Losos, 1991; Macedonia & Stamps, 1994; Kwiatkowski & Sullivan, 2002).

Our results suggest that despite a role for colour in signalling fighting ability or status in *C. vadnappa*, prior agonistic experience can have a remarkably long lasting effect that overrides the importance of colour signals. Previous studies on fish, reptiles and invertebrates have demonstrated only relatively short-term experience effects or rival recognition ranging from 1 hour to 2 days (Chase et al., 1994; Schuett, 1997; Whitehouse, 1997; Hsu & Wolf, 1999; Khazraie & Campan, 1999; Bergman et al., 2003). Studies using individuals more than once have shown that 24 to 72 h between contests is sufficient to account for any experience effects in invertebrates and fish (e.g., Hofman & Schildberger, 2001; Moretz, 2003). The longest experience effect recorded from experimental studies that we are aware of is 1-2 weeks for the lobster *Homarus americanus* (Karavanich & Atema, 1998). Results of our study suggest that in rock dragons, previous encounters can affect aggressive behaviour for up to three weeks, at least in an experimental setting where individuals have no other agonistic experiences in the intervening period. Ideally, this finding should be validated by experiments examining the effects of differing social experience and environmental context on aggressive behaviour. Nevertheless, our results have important implications for behavioural studies. Although it is always preferable to avoid using the same individuals in experiments such as these, for many taxa, obtaining and maintaining a sufficiently large number of individuals is often very difficult. Our results suggest that for taxa in which the duration of experience effects is not known, at the very least, extended periods need to be allowed between trials when using individuals more than once.

'Experience effects' may be due to a winner/loser effect whereby winning or losing affects the probability of winning or losing subsequent contests as well as their intensity and duration (e.g., Jackson, 1991; Dugatkin, 1997; Schuett, 1997; Whitehouse, 1997; Hsu & Wolf, 1999; Khazraie & Campan, 1999; Mesterton-Gibbons, 1999; Daws et al., 2002; Bergman et al., 2003; Trainor et al., 2004). Alternatively, the effect may be due to rival recognition and pre-established dominance relationships (e.g., Rosenberg & Enquist, 1991; Olsson, 1994b; Stamps & Krishnan, 1998; Whiting, 1999; Lopez & Martin, 2001). In this study, we used the same pair of lizards in the three types of trial (one natural, two manipulated), yet the appearance of the contestants changed dramatically between trials and contestants may have perceived their opponent as being different. If alteration of coloration prevented rival recognition, then the effect of contest order detected may be due to simply to a winner or loser effect. Recent experimental studies provide support for rival recognition in *C. decresii* and suggest that the basis for recognition is the unique throat colour pattern of individual males, which, when masked, prevents recognition (Osborne, 2004, in press). If this is the case for both *C. decresii* and *C. vadnappa*, then our results most likely reflect the effects of prior winning or losing experiences on agonistic behaviour as throat coloration was altered in successive trials.

The strong and long-lasting effect of previous experiences that we detected has implications for territory defence and maintenance in the wild. Male rock dragons maintain exclusive territories that they defend from neighbours and intruders. Individual recognition or alteration of fighting behaviour based on previous fighting experience may both be important ways of reducing the costs of aggression in territorial animals (Olsson, 1994b; Stamps & Krishnan, 1998; Whiting, 1999; Lopez & Martin, 2001). In lizards, as in many other taxa, aggressive behaviour may depend greatly on prior experience and established dominance relationships with neighbours (Olsson, 1992, 1994b; Whiting, 1999). However, when territory holders encounter a novel intruder, colour may be important in opponent assessment and strongly influence whether or not a contest escalates and ultimately, who wins. In addition, colour may be an important signal at greater distances, and serve to prevent encounters from escalating into fights, which are energetically expensive and may cause injury (Losos, 1985; Whiting et al., 2003).

Overall, we found evidence for long-lasting effects of previous contests on aggression levels and contest outcome of subsequent contests and that such experience effects override colour in influencing contest outcome. However, in initial contests between unfamiliar males, both the conspicuous throat and flank colours influence contest outcome and opponent assessment in *C. vadnappa*, but not *C. decresii*. In conjunction with results of previous studies (Stuart-Fox et al., 2003, 2004), this study provides experimental support for the hypothesis that colour variation in *C. vadnappa* can be explained by the trade-off between natural selection for crypsis and intrasexual selection for conspicuous coloration. This is also consistent with our finding that the throat in *C. vadnappa* appears to be more important than the flank markings in determining contest outcome and is generally concealed from avian predators except when displaying. Our study, in focussing on the role of colour in male-male contests in two species of rock dragon, begins to unravel the role of sexual selection in contributing to the striking colour variation among rock dragons. However, factors affecting aggression and contest outcome in these lizards, and their interaction, may be much more complex than previously appreciated.

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