Sexual coloration and sperm performance in the Australian painted dragon lizard, Ctenophorus pictus

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Abstract

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Keywords: sexual selection, female choice, sperm competition, precopulatory, postcopulatory, lizard, trade offs
Introduction

Sexual selection is a powerful evolutionary force that can act both before and after copulation (Birkhead and Moller, 1998) shaping the morphology, physiology and behaviour of sexually reproducing organisms (Andersson, 1994). These selective episodes are often examined in isolation (Kvarnemo & Simmons, 2013), which is problematic if we intend to capture the interacting effects of pre- and postcopulatory sexual selection on male fitness and phenotypic evolution (Lupold et al., 2014, Devigili et al., 2015, Dines et al., 2015, Marie-Orleach et al., 2016, Naretto et al., 2016, Evans & Garcia-Gonzalez, 2016). Similarly, precopulatory mate choice and intrasexual contest success are rarely tested together, and the relative strength of each varies across taxa. In reptiles, male-male contests are widely thought to drive most of the variation in male mating success, with very few known examples of reptile female mate choice (Olsson & Madsen, 1995, Olsson & Madsen, 1998, Olsson et al., 2013).

Predictive models of sperm competition and strategic ejaculate investment often assume that there is a trade-off between pre- and postcopulatory traits as a result of limited resources allocated to reproduction (Parker, 1970, Parker & Pizzari, 2010, Parker et al., 2013). Within individuals this assumption is supported by energetic costs of traits selected by postcopulatory sperm competition (i.e., sperm and seminal fluid production; e.g., Dewsbury, 1982, Olsson et al., 1997, Friesen et al., 2015) and precopulatory sexually selected traits (i.e., weapon development, display traits and associated behaviours; e.g., Allen & Levinton, 2007, Emlen, 2001, Clark, 2012). Furthermore, when precopulatory selection is strong may weaken the selection pressure on and postcopulatory traits by relaxing the intensity of sperm competition, (e.g. Taborsky, 1998, Pitnick et al., 2001, Olsson et al., 2005, Fitzpatrick et al., 2007). Alternatively, some studies demonstrate that the same individuals can have both large weapons or bright colours and competitive ejaculates (i.e. additive relationship; Evans et al., 2003, Pilastro et al., 2004, Malo et al., 2005, Locatello et al., 2006, Hosken et al., 2008), and others have found no such relationship (Pischedda & Rice, 2012, Lewis et al., 2013, Travers et al.,...
Parker et al. (2013) suggested, based on theoretical analyses, that the degree to which males are able to monopolise access to females via precopulatory sexual selection (sensu Emlen & Oring, 1977) may determine the strength and direction of this trade-off among species. Taxonomic comparisons support this argument, demonstrating that taxa in which males are able to monopolise females, males are more likely to exhibit a trade-off between precopulatory armaments and testes size (Fitzpatrick et al., 2012, Lupold et al., 2014, Dines et al., 2015, Dunn et al., 2015, Kahrl et al., 2016). Sperm performance has received minimal investigation as an important postcopulatory trait in this framework (Helfenstein et al., 2010a, Locatello et al., 2006, Pitcher et al., 2007, Rowe et al., 2010). Examination of parameters such as sperm velocity and percentage motility should provide insight into male copulatory strategies (reviewed in Simmons & Fitzpatrick, 2012, Fitzpatrick & Lupold, 2014). Such analyses would be particularly interesting in intraspecific studies on males with divergent life-history strategies (Lupold et al., 2014).

The Australian painted dragon (Ctenophorus pictus, Peters 1866) is an excellent system to test the relationship between sexual signalling traits and sperm performance. Males of this species actively patrol and defend territories and can largely monopolise access to females that inhabit their home ranges, as evidenced by >80% of clutches being sired solely by the territorial male (Olsson et al., 2007b). Males are polymorphic with respect to both head colour morph (red, yellow, orange, or blue) and the presence or absence of a yellow gular ‘bib’. Red-headed males are more aggressive and dominate yellow males in intrasexual contests (Healey et al., 2007) and yellow males do four times better than red males in controlled sperm competition trials (Olsson et al., 2009b). Orange and blue head-colour morphs have since invaded this population but have not been behaviourally characterised. Approximately 40% of males exhibit a yellow gular patch (hereafter ‘bib’; MO and CRF, 2013-2015, unpublished data). In the wild bibbed males suffer greater condition loss than non-bibbed males through the breeding season (Healey & Olsson, 2009). Importantly, bibbed males are less likely to lose paternity to their territorial neighbours than are non-bibbed males (Olsson et al., 2009a). Whether the bib plays a direct role in mediating this apparent reproductive advantage as a
sexually selected trait is unknown, however the ventral, gular placement of the bib is common among lizard signallers (Cuervo & Shine, 2007, Vercken et al., 2007, Lattanzio et al., 2014). If this is a sexual signal, we predict that the bib morphs employ reproductive strategies with different investment in pre- and postcopulatory traits, which might explain the maintenance of this polymorphism (Barlow, 1973, Tuttle, 2003, Yewers et al., 2016, Healey et al., 2007).

Here we used behavioural and sperm performance assays to test whether sexual signals and sperm performance traits trade off. We had three aims: 1) determine whether bib presence or quality (size or colouration) predicts dyadic contest success and level of aggression; 2) test whether females exhibit a preference for the presence or quality of a bib; 3) measure sperm velocity and the percentage of motile sperm as indicators of sperm performance in bibbed and non-bibbed males.

**Materials and Methods**

**Study species**

The Australian painted dragon (*Ctenophorus pictus*) is a small (adult snout-to-vent length 55-75mm, mass 8-16g) lizard found in sandy arid areas in south-central Australia (Cogger, 2014). Lizards for this study were captured at Yathong Nature Reserve, New South Wales (145°35’; 32°35’), in October and November 2015. Upon capture, we measured body size (snout-vent length; SVL to the nearest 1mm) and mass (to the nearest 0.1g), photographed and scored males by eye for head colour (red, yellow, orange or blue) and bib morph (presence/absence) (Olsson et al., 2007b, Healey & Olsson, 2009). We calculated body condition as the residuals generated by linear regression analysis of ln(mass) as a function of ln(SVL) (Schulte-Hostedde et al., 2005, Friesen et al., 2017). Within eight days of capture we re-located all animals to the University of Sydney and housed them in plastic tanks (60 x 60 x 50 cm) with a perch, ceramic hide, and a 60-W heat lamp at one end to allow for behavioural thermoregulation (Olsson et al., 2007b). Males were housed individually and (non-territorial) females were housed in pairs on a 12:12 h light regime (light: dark). Animals were fed mealworms dusted
with calcium and multivitamins *ad libitum* every second day and misted with water daily. University of Sydney Animal Care and Ethics committee approved all husbandry protocols and experiments (L04/09-2013/3/6050), and animals were collected with permission from the National Parks and Wildlife Service, New South Wales, Australia (permit SL100352).

**Female choice trials**

We conducted three consecutive trials with the same receptive female and size- and colour-morph-matched male-pair (same male pairs as used for the intrasexual contests above), one with and one without a bib. The female was pre-ovulatory and receptive based on palpation, days since last clutch, and behaviour (Healey et al., 2008, Olsson et al., 2009a). Each of the three trials were conducted in separate but identical arenas (Figure 1) within two minutes of the end of the previous trial. The two males were held in adjacent compartments where they could not see each other. A transparent plastic partition separated the males from the main area of the arena. The female was held in a compartment at the end opposite the males behind two removable partitions: one opaque and one transparent. We filmed from above using a webcam (Logitech, c615 HD) and monitored in real-time on a monitor without disturbing the animals. After 4.5 minutes of acclimation we removed the opaque partition, enabling the female and males to see each other, when the males turned to face the female (revealing their gular region). After the female observed the males facing her for 30s, the transparent partition was removed, allowing the female to approach the males’ compartments. Prior to experiments, we defined female ‘preference’ of a particular male to occur after she made contact with the transparent screen separating her from that male in at least two of the three trials. We excluded trials from analyses if, after 10 minutes, females did not choose either male, and instead only explored the arena (e.g. approached the males screen along the side of the container). A total of 14 set of three trials were completed, in which each male (n=28; 14 male pairs) and eight of the females were included once. However, three of the females were used twice, so female ID was included as a random factor in the mixed model analysis. To account for differences in male behaviour, we recorded head-bobs, although this turned out to be an infrequent behaviour that
most males failed to do. More commonly males ran against the partition trying to approach the female, quite possibly because head bobbing is predominantly used for communicating at greater distances (Fleishman, 1992).

**Male-Male Contest Behavioural Assays**

Male-male contests were staged between pairs of condition- and head-colour morph- matched males (SVL difference: $\mu=0.29\text{cm SD}=0.17$, mass difference; $\mu=0.74\text{g, SD}=0.8$), one with and one without a bib, in the presence of a receptive female (Healey et al., 2007). Contests took place in a triangular arena and were filmed both adjacently (Nikon D90 with Nikon ED lens 77) and from above (Logitech, c615 HD). Before each contest, males acclimated in opaque, removable compartments midway along two sides (side randomised), while the female spent the duration of the trial in a semi-circular mesh compartment midway along the remaining side (Figure 2). After a 3-minute acclimation period, an observer slowly raised the opaque compartments using a pulley system, exposing the males to each other directly, as well as to the female through the mesh. Males performed stereotyped, aggressive behaviour towards each other (i.e., ventro-dorsal flattening, push-ups and head-bobs; Healey et al., 2007). Trials ended when a male showed submissive behaviour (backing or running away, crouching, pressing to the ground; Healey et al., 2007). We scored the winner and loser based on these behaviours, and scored the aggressor as the male who initiated the aggressive behaviour during the contest (Whiting et al., 2003, Healey et al., 2007). We used each male in one trial only (total 11 trials, 22 unique males), and staged all male-male contest trials after the female-choice trials and sperm sampling, to avoid winner/loser effects on male behaviour towards females or sperm performance traits (winner and loser effects; Hsu et al., 2006, Oyegbile & Marler, 2005).

**Bib Size and Colouration**

We quantified bib size and colour saturation (‘chroma’) using digital photography analysed in Adobe Photoshop (San Jose, CA, USA). Digital photography, which relies on visible-light, is suitable to
capture colour variation in this species, since *C. pictus* skin does not reflect in the UV (Healey et al., 2007, Giraudeau et al., 2016, Friesen et al., 2017). At capture, we photographed each male’s throat (Nikon D810 35-300mm Nikon SWM VR ED IF Aspherical 77mm lens, Thailand) under standardised conditions (45 mm, 300 mm above the male, exposure 1/60sec, F-22). All photographs included a standard colour-board and a scale (mm; colorchecker passport, X-rite Model No. M3CPP). Colour saturation (‘chroma’) is a measure of the purity or intensity of a colour (ranging from grey to fully saturated), and is the relevant measure because bib colouration ranges from pale grey scales with a hint of faded yellow to intense yellow colouration. Saturation is also relevant to physiological investment in colour production, as it likely corresponds to colour pigment density (Pryke et al., 2001, Isaksson et al., 2006, Kemp et al., 2015). Adobe Photoshop can also provide measures of hue (the wavelengths of light reflected), and brightness (how light or dark the colouration is). These do not address our trait of interest; how intense vs faded the colour of the bib is.

In Adobe Photoshop we selected the bib area (using ‘magic wand’ tool, tolerance: 32), and on non-bibbed males we selected a small (1500-2500 pixels) area in the central gular region. Bib size was calculated by referencing the scale. To determine colour saturation for a selected area we used the ‘histogram’ tool in Photoshop to give the mean red, green and blue values, then the ‘colour picker’ tool to translate these to mean saturation (which Photoshop gives in HSB colour space; Cox et al., 2008). For each photo we calculated saturation for both the selected bib area (or non-bibbed area) and a selected area of a particular standard colour on the colour-board. The difference between the gular saturation and standard colour saturation gave standardised bib saturation that was used for statistical analyses. The photo of each male’s gular region was analysed twice and these values were highly repeatable (bib saturation $R^2=0.9861$, non-bib skin saturation $R^2=0.9758$, bib size $R^2=0.9388$; all $p<0.0001$). We used the average of these two values for statistical analyses. Averaged bib size was regressed on body size (SVL) and the residuals used to control for the effect of male size on bib size.
Bib size and saturation were highly positively correlated (R²=0.704, p=0.0002). Specifically, bibbed males either had large intensely coloured bibs, or small faded bibs, further suggesting that saturation is an appropriate measure for bib colouration. However, because some (especially larger, more saturated) bibs faded towards the edge, it was difficult to identify the biologically relevant border of measurement of the bib to accurately determine bib size. Additionally, we could not use a threshold value for saturation to exclude faded edges, because the saturation value in small ‘faded’ bibs was only equal to that on the very edges of highly saturated bibs. Thus, for statistical analysis we used saturation as the only measure of bib quality, which also avoided the issues with separately testing autocorrelated variables.

**Sperm Performance traits**

Subsequent to female-choice trials and prior to male contest trials we collected a sperm sample from each male used in the trials (n=28 males) by gently evertng the male’s hemipene, and drawing the ejaculate into a pipette with HAMS F-10 solution (Cat # 99175, Irvine Scientific, Santa Ana, CA, USA; 21 mM HEPES buffer, 4 mM sodium bicarbonate, 1 mM calcium lactate, 0.5 mM magnesium sulfate, 5 mg mL⁻¹ (0.5%) human albumin; e.g., Mattson et al., 2007, Friesen et al., 2013, Friesen et al., 2014). The ejaculate was diluted and slowly pipetted into a counting chamber slide (Hamilton-Thorne 2X-CEL®). Sperm were examined with a phase contrast compound microscope (Nikon E200) using the 4x objective. A digital camera (Basler Aviator AVA1000-100gc) mounted via a 0.70x extension tube, captured videos that were analysed using computer-assisted sperm analysis (CASA; Microptic Sperm Class Analyzer 5.4.0.0 SCA®). Sperm were tracked for 30 images in each field of view with the frame rate set at 50 frames/s. Sperm tracked for less than 10 frames were excluded. On average n= 471 sperm tracks (SD= 213.5) were analysed per individual. To control for the potential effects of drift, only sperm cells with a curvilinear (VCL) of >10µm s⁻¹ were considered motile. The cell detection parameters included the cell size of 60-200µm², and all analysed videos were scrutinised by eye to ensure non-sperm particles, such as red blood cells and faecal matter, were not selected. Also, in a few cases we deleted tracks where two sperm crossed paths and the CASA switched sperm mid-
track. Where a sperm track was interrupted and so two non-independent tracks occurred, the earlier track was deleted. We used average VAP (the average velocity over a smoothed sperm path) as our measure of sperm velocity in statistical analyses.

**Statistical Analysis**

*Female choice trials:* We tested for an association between female preference and males bib-morph (present or absent) using a Generalized Linear Mixed Model (GLMM, binomial distribution, logit link function using SPSS version 22, IBM) with female preference as a binary response variable, bib presence or absence as a fixed effect and female ID as a random effect (three of eleven males were used twice). Because the males were size- (± 0.29 mm) and mass- (± 0.74 g) matched, it is not surprising that preliminary analyses showed that body size (SVL) and body condition were not significantly (p<0.25) associated with female preference, so we excluded them from the final model. We also tested whether gular saturation (or bib quality) was associated with female preference using a GLMM (binomial distribution, logit link function) with female preference as the response variable, saturation as a fixed effect, and female ID as a random effect. Again body size and condition were excluded from the final model. With small sample sizes overdispersion can be a problem, so to ensure our results were not affected by liberal standard errors, we tested for overdispersion of our final model in R (R Development Core Team, 2010; see script uploaded to Dryad). We performed a t-test to confirm that bibbed males had higher saturation than non-bibbed males (data met the assumptions of normality and homogeneity of variance).

*Dyadic contests:* We performed a test of independence (chi-square distribution) to examine whether bibbed males won more contests than non-bibbed males. To determine whether males that initiated aggression won more contests we performed a test of independence using Monte Carlo simulations to estimate Chi-square distributions and p-values because there were structural zeros in the data set (aggressor males always won) using XLSTAT Version 2014.1.08, Addsoft™. We tested whether yellow throat saturation predicted contest success using a GLMM (binomial distribution, logit link function,
Satterthwaite approximation for degrees of freedom because of small sample sizes) with contest success as a binary response variable, saturation as a fixed effect, and trial number as a random effect (to account for males being paired: one lost the other won). Robust estimations of fixed effect and coefficients to handle slight violations of model assumptions. Preliminary analyses included body size (SVL) and body condition, but were not significant (p>$0.25$) again due to size matching as part of the experimental design, so were dropped from the model. We also tested whether gular saturation differed between winners and losers using a paired t-test.

**Sperm performance:** We performed t-tests in SPSS to determine whether the percent of motile sperm differed between bibbed males and non-bibbed males. We used an Independent-Samples Mann-Whitney U-Test to compare VAP between bibbed and non-bibbed males, as these data could not be transformed to normality (SPSS). We used GLM (SPSS) to test for associations between gular saturation, percent motile sperm and sperm velocity (VAP). We performed separate regression analyses to investigate whether there was a relationship between VAP and percent motile, and whether there was a relationship between body condition and either of these sperm performance measures.

**Results**

**Female choice trials:** Females were more likely to associate with bibbed males than non-bibbed males (females chose bibbed males in 10 of 14 trials, GLMM: $F_{1,26}=4.766$, $p=0.038$, Figure 3). This GLMM did not suffer significant overdispersion ($X^2_{28}=28$, rdf=25, $p=0.31$). As expected, bibbed males had higher gular saturation than non-bibbed males ($t_{26}=7.033$, $p<0.001$), however colour saturation was not associated with female preference ($F_{1,26}=1.113$, $p=0.301$). Body condition did not differ between bibbed and non-bibbed males ($t_{26}=0.241$, $p = 0.679$) and was not associated with gular saturation ($r=0.045$, N=28, $p=0.821$).

**Dyadic contests:** The male who displayed the first aggressive behaviour won in every trial ($X^2_{1}=11.00$, $P < 0.003$). Bibbed males won 7 contests and non-bibbed males won 4 contests, which is not
significantly different ($X^2_{\text{df.1}} = 1.636, P = 0.201$; Figure 3). However, unlike female preference trials, the likelihood of male contest success was influenced by gular saturation (GLMM: $F_{1,20}=5.352$, $p=0.031$). This GLMM did not suffer significant overdispersion ($X^2=20.76$, rdf=19, $p=0.35$). The importance of variation in throat colouration for contest outcomes was confirmed by gular saturation differing significantly between winners and losers ($t_{10}=2.415$, $p=0.036$).

**Sperm performance:** The presence of a bib did not predict sperm performance as measured by the percentage of motile sperm ($t_{26}= 0.423$, $p=0.676$, mean difference = -1.53, SE of difference ±3.62) or sperm velocity (VAP; N=28, Mann-Whitney U = 90, $p=0.713$, standardised test statistic = -0.368). Gular saturation also did not predict either measure of sperm performance (percentage of motile sperm: $r =0.055$, N=28, $p=0.782$; sperm velocity: VAP, $r=0.015$, N=28, $p=0.939$). There was a significant relationship between percentage motile sperm and VAP across males ($R^2 = 0.4636$, $F_{1,26} = 22.476$, $p<0.001$). Body condition did not predict percentage motile sperm ($R^2 = 0.0005$, $F_{1,26} = 0.023$, $p = 0.88$) or VAP ($R^2 = 0.00327$, $F_{1,26} = 0.23$, $p = 0.64$).

**Discussion**

We tested for a precopulatory sexually selected advantage for males with a colourful gular bib and then tested for covariation between this trait and sperm performance as evidence for a trade-off between pre- and postcopulatory traits (Lupold et al., 2014, Parker et al., 2013). Previous evidence that bibbed males are more likely to sire sole-paternity clutches in the wild (Olsson et al., 2009a) suggests that they may be better able to monopolise females or are superior sperm competitors. Our behavioural assays support two possible, non-mutually exclusive mechanisms for mate monopolization: 1) bib quality was associated with aggressiveness and contest success, which are both likely to be important in territorial defense (Emlen & Oring, 1977, Shuster & Wade, 2003, Whiting et al., 2003), and 2) females preferred bibbed males, which could influence the rate that females seek out extra-territorial copulation similar to some socially monogamous birds (Kempenaers et al., 1992, Ratti et al., 1995, Andersson & Simmons, 2006). Although bibbed and non-
bibbed males did not differ in sperm performance, there was significant among male variation and
within males and there was a positive relationship between sperm velocity (VAP) and the percentage
of motile sperm suggesting both measures indicate postcopulatory trait quality.

Precopulatory sexual selection

Our study is significant because reptilian mate choice appears rare, particularly for colour traits
identified example is that female side-blotched lizards (Uta stansburiana) exert mate preference for a
particular dorsal pattern morph (a polymorphic antipredator trait), but only specifically in
combination with males of the yellow throat (sneaker) morph (Lancaster et al., 2009). If female
painted dragons (Ctenophorus pictus) are capable of similarly sophisticated mate preferences, then
the strength of female preference for bibbed males may vary with male head colour morph (and
corresponding reproductive strategy; Healey et al., 2007, Olsson et al., 2009b, Olsson et al., 2007a)
for optimal trait combinations. Our study also provides further support that aggression is important
for reptile intrasexual contest outcomes and can be signalled by colour traits (Olsson & Madsen,

Trade-off: pre- vs. postcopulatory traits?

Under the assumption of (high) costs of sexual colour signals, our results lend little support to the
assumptions of sperm competition modelling (Parker et al., 2013); we did not find evidence that
males with bibs had lower sperm performance. This is surprising, as we might expect weaker
selection on ejaculate performance in bibbed males. However, current sperm performance
characteristics may reflect selection pressures of the recent past such that shifts in bib frequency
over evolutionary time change selection dynamics, with bibbed males still facing sperm competition
at high population bib frequency. We also expect bibbed males to have fewer resources for investing
in ejaculate quality (Kotiaho, 2001, Blount, 2004). The bib is likely carotenoid based, as male painted
dragons are known to use carotenoids to produce yellow head colouration (Olsson et al., 2008, Olsson et al., 2012), and carotenoid-pigment is widely considered costly due to environmental availability and investment trade-offs (e.g. antioxidant activity or immune function; Olson & Owens, 1998, Svensson & Wong, 2011). Instead of sperm performance traits, the energetic allocation trade-off with bib expression may be with investment into other life-history traits such as somatic investment, reactive oxygen production, antioxidant capacity or growth rate between bib-morphs (e.g. Thompson et al., 1993, Dijkstra et al., 2016). Frequent aggressive activity by bibbed males may also provide a mechanistic link to these, as aggression and territorial patrolling behaviour may increase energetic costs, stress and testosterone-mediated immunosuppression, and oxidative stress (Folstad & Karter, 1992, Whiting et al., 2003, Briffa & Sneddon, 2007, Clark, 2012, Sharick et al., 2015, Costantini, 2014). Previous data supports this possibility as bibbed males lose body condition at a greater rate than non-bibbed males when under physiological or social stress in the wild (Olsson et al., 2009a, Healey & Olsson, 2009).

An allocation trade-off between bib expression and other life history traits may contribute to polymorphism maintenance, as if bibbed males have a precopulatory advantage and all else was equal we would expect the bib phenotype to come to fixation (McKinnon & Pierotti, 2010). An allocation trade-off between bib expression and other life-history traits may well act in combination with frequency-dependent selection to maintain this polymorphic trait, as occurs in the Gouldian finch Erythrura gouldiae. In this polymorphic finch, when exposed to high frequencies of red (aggressive) morph males, red males experience a more intense stress response and greater immunosuppressive reaction than subordinate (black) males (Pryke et al., 2007). When they reach high frequency in the population red males thus experience this greater physiological cost, causing their frequency in the population to decrease, facilitating frequency-dependent selection to maintain this polymorphism (Pryke et al., 2007). In addition to potentially explaining the maintenance of the bib polymorphism, bibbed males trading-off a precopulatory sexually selected advantage with other life-history traits draws attention to and questions one of the underlying assumptions for an
energetic trade-off between pre- and postcopulatory traits: that a certain energy allocation is made to reproductive traits and then subdivided between pre- and postcopulatory traits.

It is feasible that there is a trade-off between bib expression and sperm performance (and body condition and sperm performance) that this study failed to find. Males in this study had ad libitum food resources, reduced activity levels (no territory roaming or fighting), and fewer, or absence of, ectoparasites (in captivity), which may mask condition-dependent trade-offs. For example, in male *Anolis sagrei* body condition that varies naturally or due to dietary restriction corresponds to differences in sperm morphology, and high condition males have greater success in competitive fertilisation trials (Kahrl & Cox, 2015). It is also possible that the lack of difference we observed between bib morphs is due to somewhat low power for sperm traits (n=28 males). Furthermore, there is already evidence that different head colour morphs differ in postcopulatory traits such as testes size and success in sperm competition trials, and strong differences between them could be masking sperm differences due to bib morph. While balanced for bib morphs, our sample sizes differed among head colour morphs (between 2 and 12 males of each). However, when included as an explanatory factor in our model, head colour did not explain significant amount of variance, although we had low power, and would require much larger and more balanced samples to detect a head-morph effect.

**Sperm performance**

Sperm velocity and percentage of motile sperm are important postcopulatory traits across a number of internally fertilising taxa (Møller, 1988, Firman & Simmons, 2010, Simmons & Fitzpatrick, 2012), however to our knowledge this has not been tested in a reptile species, so it is possible that other postcopulatory traits, such as longevity, are more influential for fertilisation success. Female *C. pictus* can store sperm from multiple males in their reproductive tract for extended periods of time (Olsson et al., 2009b). There is therefore likely a selective advantage for sperm longevity (Dziminski et al., 2009, Fitzpatrick et al., 2009), which may even be inversely related to sperm velocity (Levitan, 2000,

**Conclusion**

In conclusion, the saturation of bib colour predicted contest success, and females preferentially associated with bibbed over non-bibbed males. Placed in the context of prior research, we demonstrate the importance of this polymorphic colour trait as a precopulatory sexual signal, but found no evidence for a trade-off with sperm quality. We suggest that it would be valuable to test this hypothesis again in the wild with additional measures of postcopulatory quality, as condition dependence may be driving this proposed trade-off.

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**Figure Legends**

**Figure 1.** Diagrammatic representation of the arenas during the three stages of each female choice trial, with two condition- and colour-morph matched males, one with a bib (B) and one with no bib (NB) randomly assigned a side, and a receptive female. A) Acclimation period, visibility between all lizards is blocked. B) Female visually exposed to the two males. C) Female
can approach males. Trial finishes if female makes a ‘choice’, defined as her head contacting the transparent screen in front of a male’s compartment. Trials are then performed twice more, with males switching sides each trial. Female ‘prefers’ a male she chooses at least two times.

Figure 2. Diagrammatic representation of the arena in which the staged contest trials were performed. After a 3-minute acclimation period male compartments were removed, exposing the males to each other directly, and to the receptive female through the mesh.

Figure 3. Number of times the receptive female preferred (chose in at least two of three consecutive trials) bibbed and non-bibbed males. The number of staged contests won by bibbed and non-bibbed males.

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