

University of Wollongong

Research Online

---

Faculty of Science, Medicine and Health -  
Papers: Part B

Faculty of Science, Medicine and Health

---

1-1-2017

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

C McDiarmid  
*University of Sydney*

Christopher R. Friesen  
*University of Wollongong, cfriesen@uow.edu.au*

C Ballen  
*University of Minnesota*

Mats M. Olsson  
*University of Wollongong, molsson@uow.edu.au*

Follow this and additional works at: <https://ro.uow.edu.au/smhpapers1>

---

### Publication Details Citation

McDiarmid, C., Friesen, C. R., Ballen, C., & Olsson, M. M. (2017). Sexual coloration and sperm performance in the Australian painted dragon lizard, *Ctenophorus pictus*. Faculty of Science, Medicine and Health - Papers: Part B. Retrieved from <https://ro.uow.edu.au/smhpapers1/986>

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: [research-pubs@uow.edu.au](mailto:research-pubs@uow.edu.au)

---

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

### Abstract

Theory predicts trade-offs between pre- and post-copulatory sexually selected traits. This relationship may be mediated by the degree to which males are able to monopolize access to females, as this will place an upper limit on the strength of post-copulatory selection. Furthermore, traits that aid in mate monopolization may be costly to maintain and may limit investment in post-copulatory traits, such as sperm performance. Australian painted dragons are polymorphic for the presence or absence of a yellow gular patch ('bibs'), which may aid them to monopolize access to females. Previous work has shown that there are physiological costs of carrying this bib (greater loss of body condition in the wild). Here, we show that male painted dragons use this bright yellow bib as both an inter- and intrasexual signal, and we assess whether this signal is traded off against sperm performance within the same individuals. We found no relationship between aspects of bib colour and sperm swimming velocity or percentage of motile sperm and suggest that the bib polymorphism may be maintained by complex interactions between physiological or life-history traits including other sperm or ejaculate traits and environmental influences.

### Publication Details

McDiarmid, C. S., Friesen, C. R., Ballen, C. & Olsson, M. (2017). Sexual coloration and sperm performance in the Australian painted dragon lizard, *Ctenophorus pictus*. *Journal of Evolutionary Biology*, 30 (7), 1303-1312.

1 Sexual colouration and sperm performance in the Australian  
2 painted dragon lizard, *Ctenophorus pictus*

3

4 **Abstract**

5 Theory predicts trade-offs between pre- and postcopulatory sexually selected traits. This relationship  
6 may be mediated by the degree to which males are able to monopolise access to females, as this will  
7 place an upper limit on the strength of postcopulatory selection. Furthermore, traits that aid in mate  
8 monopolization may be costly to maintain and may limit investment in postcopulatory traits, such as  
9 sperm performance. Australian painted dragons are polymorphic for the presence or absence of a  
10 yellow gular patch ('bibs'), which may aid them to monopolise access to females. Previous work has  
11 shown that there are physiological costs of carrying this bib (greater loss of body condition in the  
12 wild). Here we show that male painted dragons use this bright yellow bib as both an inter- and intra-  
13 sexual signal, and we assess whether this signal is traded off against sperm performance within the  
14 same individuals. We found no relationship between aspects of bib colour and sperm swimming  
15 velocity or percentage of motile sperm, and suggest that the bib polymorphism may be maintained  
16 by complex interactions between physiological or life-history traits including other sperm or  
17 ejaculate traits and environmental influences.

18 **Keywords:** sexual selection, female choice, sperm competition, precopulatory, postcopulatory, lizard,  
19 trade offs

## 20 Introduction

21 Sexual selection is a powerful evolutionary force that can act both before and after copulation  
22 (Birkhead and Moller, 1998) shaping the morphology, physiology and behaviour of sexually  
23 reproducing organisms (Andersson, 1994). These selective episodes are often examined in isolation  
24 (Kvarnemo & Simmons, 2013), which is problematic if we intend to capture the interacting effects of  
25 pre- and postcopulatory sexual selection on male fitness and phenotypic evolution (Lupold et al.,  
26 2014, Devigili et al., 2015, Dines et al., 2015, Marie-Orleach et al., 2016, Naretto et al., 2016, Evans &  
27 Garcia-Gonzalez, 2016). Similarly, precopulatory mate choice and intrasexual contest success are  
28 rarely tested together, and the relative strength of each varies across taxa. In reptiles, male-male  
29 contests are widely thought to drive most of the variation in male mating success, with very few  
30 known examples of reptile female mate choice (Olsson & Madsen, 1995, Olsson & Madsen, 1998,  
31 Olsson et al., 2013).

32 Predictive models of sperm competition and strategic ejaculate investment often assume that there  
33 is a trade-off between pre- and postcopulatory traits as a result of limited resources allocated to  
34 reproduction (Parker, 1970, Parker & Pizzari, 2010, Parker et al., 2013). Within individuals this  
35 assumption is supported by energetic costs of traits selected by postcopulatory sperm competition  
36 (i.e., sperm and seminal fluid production; e.g., Dewsbury, 1982, Olsson et al., 1997, Friesen et al.,  
37 2015) and precopulatory sexually selected traits (i.e., weapon development, display traits and  
38 associated behaviours; e.g., Allen & Levinton, 2007, Emlen, 2001, Clark, 2012). Furthermore, when  
39 precopulatory selection is strong may weaken the selection pressure on and postcopulatory traits by  
40 relaxing the intensity of sperm competition, (e.g. Taborsky, 1998, Pitnick et al., 2001, Olsson et al.,  
41 2005, Fitzpatrick et al., 2007). Alternatively, some studies demonstrate that the same individuals can  
42 have both large weapons or bright colours and competitive ejaculates (i.e. additive relationship;  
43 Evans et al., 2003, Pilastro et al., 2004, Malo et al., 2005, Locatello et al., 2006, Hosken et al., 2008),  
44 and others have found no such relationship (Pischedda & Rice, 2012, Lewis et al., 2013, Travers et al.,

45 2016). Parker et al. (2013) suggested, based on theoretical analyses, that the degree to which males  
46 are able to monopolise access to females via precopulatory sexual selection (sensu Emlen & Oring,  
47 1977) may determine the strength and direction of this trade-off among species. Taxonomic  
48 comparisons support this argument, demonstrating that taxa in which males are able to monopolise  
49 females, males are more likely to exhibit a trade-off between precopulatory armaments and testes  
50 size (Fitzpatrick et al., 2012, Lupold et al., 2014, Dines et al., 2015, Dunn et al., 2015, Kahrl et al.,  
51 2016). Sperm performance has received minimal investigation as an important postcopulatory trait in  
52 this framework (Helfenstein et al., 2010a, Locatello et al., 2006, Pitcher et al., 2007, Rowe et al.,  
53 2010). Examination of parameters such as sperm velocity and percentage motility should provide  
54 insight into male copulatory strategies (reviewed in Simmons & Fitzpatrick, 2012, Fitzpatrick &  
55 Lupold, 2014). Such analyses would be particularly interesting in intraspecific studies on males with  
56 divergent life-history strategies (Lupold et al., 2014).

57 The Australian painted dragon (*Ctenophorus pictus*, Peters 1866) is an excellent system to test  
58 the relationship between sexual signalling traits and sperm performance. Males of this species  
59 actively patrol and defend territories and can largely monopolise access to females that inhabit their  
60 home ranges, as evidenced by >80% of clutches being sired solely by the territorial male (Olsson et  
61 al., 2007b). Males are polymorphic with respect to both head colour morph (red, yellow, orange, or  
62 blue) and the presence or absence of a yellow gular 'bib'. Red-headed males are more aggressive and  
63 dominate yellow males in intrasexual contests (Healey et al., 2007) and yellow males do four times  
64 better than red males in controlled sperm competition trials (Olsson et al., 2009b). Orange and blue  
65 head-colour morphs have since invaded this population but have not been behaviourally  
66 characterised. Approximately 40% of males exhibit a yellow gular patch (hereafter 'bib' ; MO and  
67 CRF, 2013-2015, unpublished data). In the wild bibbed males suffer greater condition loss than non-  
68 bibbed males through the breeding season (Healey & Olsson, 2009). Importantly, bibbed males are  
69 less likely to lose paternity to their territorial neighbours than are non-bibbed males (Olsson et al.,  
70 2009a). Whether the bib plays a direct role in mediating this apparent reproductive advantage as a

71 sexually selected trait is unknown, however the ventral, gular placement of the bib is common  
72 among lizard signallers (Cuervo & Shine, 2007, Vercken et al., 2007, Lattanzio et al., 2014). If this is a  
73 sexual signal, we predict that the bib morphs employ reproductive strategies with different  
74 investment in pre- and postcopulatory traits, which might explain the maintenance of this  
75 polymorphism (Barlow, 1973, Tuttle, 2003, Yewers et al., 2016, Healey et al., 2007).

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

## 81 Materials and Methods

### 82 ***Study species***

83 The Australian painted dragon (*Ctenophorus pictus*) is a small (adult snout-to-vent length 55-75mm,  
84 mass 8-16g) lizard found in sandy arid areas in south-central Australia (Cogger, 2014). Lizards for this  
85 study were captured at Yathong Nature Reserve, New South Wales (145°35'; 32°35'), in October and  
86 November 2015. Upon capture, we measured body size (snout-vent length; SVL to the nearest 1mm)  
87 and mass (to the nearest 0.1g), photographed and scored males by eye for head colour (red, yellow,  
88 orange or blue) and bib morph (presence/absence) (Olsson et al., 2007b, Healey & Olsson, 2009). We  
89 calculated body condition as the residuals generated by linear regression analysis of  $\ln(\text{mass})$  as a  
90 function of  $\ln(\text{SVL})$  (Schulte-Hostedde et al., 2005, Friesen et al., 2017). Within eight days of capture  
91 we re-located all animals to the University of Sydney and housed them in plastic tanks (60 x 60 x 50  
92 cm) with a perch, ceramic hide, and a 60-W heat lamp at one end to allow for behavioural  
93 thermoregulation (Olsson et al., 2007b). Males were housed individually and (non-territorial) females  
94 were housed in pairs on a 12:12 h light regime (light: dark). Animals were fed mealworms dusted

95 with calcium and multivitamins *ad libitum* every second day and misted with water daily. University  
96 of Sydney Animal Care and Ethics committee approved all husbandry protocols and experiments  
97 (L04/09-2013/3/6050), and animals were collected with permission from the National Parks and  
98 Wildlife Service, New South Wales, Australia (permit SL100352).

### 99 ***Female choice trials***

100 We conducted three consecutive trials with the same receptive female and size- and colour-morph-  
101 matched male-pair (same male pairs as used for the intrasexual contests above), one with and one  
102 without a bib. The female was pre-ovulatory and receptive based on palpation, days since last clutch,  
103 and behaviour (Healey et al., 2008, Olsson et al., 2009a). Each of the three trials were conducted in  
104 separate but identical arenas (Figure 1) within two minutes of the end of the previous trial. The two  
105 males were held in adjacent compartments where they could not see each other. A transparent  
106 plastic partition separated the males from the main area of the arena. The female was held in a  
107 compartment at the end opposite the males behind two removable partitions: one opaque and one  
108 transparent. We filmed from above using a webcam (Logitech, c615 HD) and monitored in real-time  
109 on a monitor without disturbing the animals. After 4.5 minutes of acclimation we removed the  
110 opaque partition, enabling the female and males to see each other, when the males turned to face  
111 the female (revealing their gular region). After the female observed the males facing her for 30s, the  
112 transparent partition was removed, allowing the female to approach the males' compartments. Prior  
113 to experiments, we defined female 'preference' of a particular male to occur after she made contact  
114 with the transparent screen separating her from that male in at least two of the three trials. We  
115 excluded trials from analyses if, after 10 minutes, females did not choose either male, and instead  
116 only explored the arena (e.g. approached the males screen along the side of the container). A total of  
117 14 set of three trials were completed, in which each male (n=28; 14 male pairs) and eight of the  
118 females were included once. However, three of the females were used twice, so female ID was  
119 included as a random factor in the mixed model analysis. To account for differences in male  
120 behaviour, we recorded head-bobs, although this turned out to be an infrequent behaviour that

121 most males failed to do. More commonly males ran against the partition trying to approach the  
122 female, quite possibly because head bobbing is predominantly used for communicating at greater  
123 distances (Fleishman, 1992).

#### 124 ***Male-Male Contest Behavioural Assays***

125 Male-male contests were staged between pairs of condition- and head-colour morph- matched  
126 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  
127 without a bib, in the presence of a receptive female (Healey et al., 2007). Contests took place in a  
128 triangular arena and were filmed both adjacently (Nikon D90 with Nikon ED lens 77) and from above  
129 (Logitech, c615 HD). Before each contest, males acclimated in opaque, removable compartments  
130 midway along two sides (side randomised), while the female spent the duration of the trial in a semi-  
131 circular mesh compartment midway along the remaining side (Figure 2). After a 3-minute acclimation  
132 period, an observer slowly raised the opaque compartments using a pulley system, exposing the  
133 males to each other directly, as well as to the female through the mesh. Males performed  
134 stereotyped, aggressive behaviour towards each other (i.e., ventro-dorsal flattening, push-ups and  
135 head-bobs; Healey et al., 2007). Trials ended when a male showed submissive behaviour (backing or  
136 running away, crouching, pressing to the ground; Healey et al., 2007). We scored the winner and  
137 loser based on these behaviours, and scored the aggressor as the male who initiated the aggressive  
138 behaviour during the contest (Whiting et al., 2003, Healey et al., 2007). We used each male in one  
139 trial only (total 11 trials, 22 unique males), and staged all male-male contest trials after the female-  
140 choice trials and sperm sampling, to avoid winner/loser effects on male behaviour towards females  
141 or sperm performance traits (winner and loser effects; Hsu et al., 2006, Oyegbile & Marler, 2005).

142

#### 143 ***Bib Size and Colouration***

144 We quantified bib size and colour saturation ('chroma') using digital photography analysed in Adobe  
145 Photoshop (San Jose, CA, USA). Digital photography, which relies on visible-light, is suitable to



146 capture colour variation in this species, since *C. pictus* skin does not reflect in the UV (Healey et al.,  
147 2007, Giraudeau et al., 2016, Friesen et al., 2017). At capture, we photographed each male's throat  
148 (Nikon D810 35-300mm Nikon SWM VR ED IF Aspherical 77mm lens, Thailand) under standardised  
149 conditions (45 mm, 300 mm above the male, exposure 1/60sec, F-22). All photographs included a  
150 standard colour-board and a scale (mm; colorchecker passport, X-rite Model No. MSCCPP). Colour  
151 saturation ('chroma') is a measure of the purity or intensity of a colour (ranging from grey to fully  
152 saturated), and is the relevant measure because bib colouration ranges from pale grey scales with a  
153 hint of faded yellow to intense yellow colouration. Saturation is also relevant to physiological  
154 investment in colour production, as it likely corresponds to colour pigment density (Pryke et al.,  
155 2001, Isaksson et al., 2006, Kemp et al., 2015). Adobe Photoshop can also provide measures of hue  
156 (the wavelengths of light reflected), and brightness (how light or dark the colouration is). These do  
157 not address our trait of interest; how intense vs faded the colour of the bib is.

158 In Adobe Photoshop we selected the bib area (using 'magic wand' tool, tolerance: 32), and on non-  
159 bibbed males we selected a small (1500-2500 pixels) area in the central gular region. Bib size was  
160 calculated by referencing the scale. To determine colour saturation for a selected area we used the  
161 'histogram' tool in Photoshop to give the mean red, green and blue values, then the 'colour picker'  
162 tool to translate these to mean saturation (which Photoshop gives in HSB colour space; Cox et al.,  
163 2008). For each photo we calculated saturation for both the selected bib area (or non-bibbed area)  
164 and a selected area of a particular standard colour on the colour-board. The difference between the  
165 gular saturation and standard colour saturation gave standardised bib saturation that was used for  
166 statistical analyses. The photo of each male's gular region was analysed twice and these values were  
167 highly repeatable (bib saturation  $R^2=0.9861$ , non-bib skin saturation  $R^2=0.9758$ , bib size  $R^2=0.9388$ ; all  
168  $p<0.0001$ ). We used the average of these two values for statistical analyses. Averaged bib size was  
169 regressed on body size (SVL) and the residuals used to control for the effect of male size on bib size.

170 Bib size and saturation were highly positively correlated ( $R^2=0.704$ ,  $p=0.0002$ ). Specifically,  
171 bibbed males either had large intensely coloured bibs, or small faded bibs, further suggesting that  
172 saturation is an appropriate measure for bib colouration. However, because some (especially larger,  
173 more saturated) bibs faded towards the edge, it was difficult to identify the biologically relevant  
174 border of measurement of the bib to accurately determine bib size. Additionally, we could not use a  
175 threshold value for saturation to exclude faded edges, because the saturation value in small 'faded'  
176 bibs was only equal to that on the very edges of highly saturated bibs. Thus, for statistical analysis we  
177 used saturation as the only measure of bib quality, which also avoided the issues with separately  
178 testing autocorrelated variables.

### 179 ***Sperm Performance traits***

180 Subsequent to female-choice trials and prior to male contest trials we collected a sperm sample from  
181 each male used in the trials ( $n=28$  males) by gently everting the male's hemipene, and drawing the  
182 ejaculate into a pipette with HAMS F-10 solution (Cat # 99175, Irvine Scientific, Santa Ana, CA, USA;  
183 21 mM HEPES buffer, 4 mM sodium bicarbonate, 1 mM calcium lactate, 0.5 mM magnesium sulfate,  
184 5 mg mL<sup>-1</sup> (0.5%) human albumin; e.g., Mattson et al., 2007, Friesen et al., 2013, Friesen et al.,  
185 2014). The ejaculate was diluted and slowly pipetted into a counting chamber slide (Hamilton-Thorne  
186 2X-CEL®). Sperm were examined with a phase contrast compound microscope (Nikon E200) using the  
187 4x objective. A digital camera (Basler Aviator AVA1000-100gc) mounted via a 0.70x extension tube,  
188 captured videos that were analysed using computer-assisted sperm analysis (CASA; Microptic Sperm  
189 Class Analyzer 5.4.0.0 SCA®). Sperm were tracked for 30 images in each field of view with the frame  
190 rate set at 50 frames/s. Sperm tracked for less than 10 frames were excluded. On average  $n= 471$   
191 sperm tracks ( $SD= 213.5$ ) were analysed per individual. To control for the potential effects of drift,  
192 only sperm cells with a curvilinear (VCL) of  $>10\mu\text{m s}^{-1}$  were considered motile. The cell detection  
193 parameters included the cell size of  $60\text{-}200\mu\text{m}^2$ , and all analysed videos were scrutinised by eye to  
194 ensure non-sperm particles, such as red blood cells and faecal matter, were not selected. Also, in a  
195 few cases we deleted tracks where two sperm crossed paths and the CASA switched sperm mid-

196 track. Where a sperm track was interrupted and so two non-independent tracks occurred, the earlier  
197 track was deleted. We used average VAP (the average velocity over a smoothed sperm path) as our  
198 measure of sperm velocity in statistical analyses.

### 199 ***Statistical Analysis***

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

215 **Dyadic contests:** We performed a test of independence (chi-square distribution) to examine whether  
216 bibbed males won more contests than non-bibbed males. To determine whether males that initiated  
217 aggression won more contests we performed a test of independence using Monte Carlo simulations  
218 to estimate Chi-square distributions and p-values because there were structural zeros in the data set  
219 (aggressor males always won) using XLSTAT Version 2014.1.08, Addsoft™. We tested whether yellow  
220 throat saturation predicted contest success using a GLMM (binomial distribution, logit link function,

221 Satterthwaite approximation for degrees of freedom because of small sample sizes) with contest  
222 success as a binary response variable, saturation as a fixed effect, and trial number as a random  
223 effect (to account for males being paired: one lost the other won). Robust estimations of fixed effect  
224 and coefficients to handle slight violations of model assumptions. Preliminary analyses included body  
225 size (SVL) and body condition, but were not significant ( $p > 0.25$ ) again due to size matching as part of  
226 the experimental design, so were dropped from the model. We also tested whether gular saturation  
227 differed between winners and losers using a paired t-test.

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

## 236 Results

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

244 **Dyadic contests:** The male who displayed the first aggressive behaviour won in every trial ( $X^2_{\text{d.f. 1}} =$   
245  $11.00$ ,  $P < 0.003$ ). Bibbed males won 7 contests and non-bibbed males won 4 contests, which is not

246 significantly different ( $X^2_{d.f.1} = 1.636$ ,  $P = 0.201$ ; Figure 3). However, unlike female preference trials,  
247 the likelihood of male contest success was influenced by gular saturation (GLMM:  $F_{1,20}=5.352$ ,  
248  $p=0.031$ ). This GLMM did not suffer significant overdispersion ( $X^2=20.76$ ,  $rd=19$ ,  $p=0.35$ ). The  
249 importance of variation in throat colouration for contest outcomes was confirmed by gular  
250 saturation differing significantly between winners and losers ( $t_{10}=2.415$ ,  $p=0.036$ ).

251 **Sperm performance:** The presence of a bib did not predict sperm performance as measured by the  
252 percentage of motile sperm ( $t_{26}= 0.423$ ,  $p=0.676$ , mean difference =  $-1.53$ , SE of difference  $\pm 3.62$ ) or  
253 sperm velocity (VAP;  $N=28$ , Mann-Whitney  $U = 90$ ,  $p=0.713$ , standardised test statistic =  $-0.368$ ).  
254 Gular saturation also did not predict either measure of sperm performance (percentage of motile  
255 sperm:  $r = 0.055$ ,  $N=28$ ,  $p=0.782$ ; sperm velocity: VAP,  $r_s=0.015$ ,  $N=28$ ,  $p=0.939$ ). There was a  
256 significant relationship between percentage motile sperm and VAP across males ( $R^2 = 0.4636$ ,  $F_{1,26} =$   
257  $22.476$ ,  $p<0.001$ ). Body condition did not predict percentage motile sperm ( $R^2 = 0.0005$ ,  $F_{1,26} = 0.023$ ,  
258  $p = 0.88$ ) or VAP ( $R^2 = 0.00327$ ,  $F_{1,26} = 0.23$ ,  $p = 0.64$ ).

## 259 Discussion

260 We tested for a precopulatory sexually selected advantage for males with a colourful gular bib and  
261 then tested for covariation between this trait and sperm performance as evidence for a trade-off  
262 between pre- and postcopulatory traits (Lupold et al., 2014, Parker et al., 2013). Previous evidence  
263 that bibbed males are more likely to sire sole-paternity clutches in the wild (Olsson et al., 2009a)  
264 suggests that they may be better able to monopolise females or are superior sperm competitors. Our  
265 behavioural assays support two possible, non-mutually exclusive mechanisms for mate  
266 monopolization: 1) bib quality was associated with aggressiveness and contest success, which are  
267 both likely to be important in territorial defense (Emlen & Oring, 1977, Shuster & Wade, 2003,  
268 Whiting et al., 2003), and 2) females preferred bibbed males, which could influence the rate that  
269 females seek out extra-territorial copulation similar to some socially monogamous birds  
270 (Kempnaers et al., 1992, Ratti et al., 1995, Andersson & Simmons, 2006). Although bibbed and non-

271 bibbed males did not differ in sperm performance, there was significant among male variation and  
272 within males and there was a positive relationship between sperm velocity (VAP) and the percentage  
273 of motile sperm suggesting both measures indicate postcopulatory trait quality.

#### 274 **Precopulatory sexual selection**

275 Our study is significant because reptilian mate choice appears rare, particularly for colour traits  
276 (Olsson & Madsen, 1995, Olsson & Madsen, 1998, Tokarz, 1995, Olsson et al., 2013). One previously  
277 identified example is that female side-blotched lizards (*Uta stansburiana*) exert mate preference for a  
278 particular dorsal pattern morph (a polymorphic antipredator trait), but only specifically in  
279 combination with males of the yellow throat (sneaker) morph (Lancaster et al., 2009). If female  
280 painted dragons (*Ctenophorus pictus*) are capable of similarly sophisticated mate preferences, then  
281 the strength of female preference for bibbed males may vary with male head colour morph (and  
282 corresponding reproductive strategy; Healey et al., 2007, Olsson et al., 2009b, Olsson et al., 2007a)  
283 for optimal trait combinations. Our study also provides further support that aggression is important  
284 for reptile intrasexual contest outcomes and can be signalled by colour traits (Olsson & Madsen,  
285 1998, Uller & Olsson, 2008).

#### 286 **Trade-off: pre- vs. postcopulatory traits?**

287 Under the assumption of (high) costs of sexual colour signals, our results lend little support to the  
288 assumptions of sperm competition modelling (Parker et al., 2013); we did not find evidence that  
289 males with bibs had lower sperm performance. This is surprising, as we might expect weaker  
290 selection on ejaculate performance in bibbed males. However, current sperm performance  
291 characteristics may reflect selection pressures of the recent past such that shifts in bib frequency  
292 over evolutionary time change selection dynamics, with bibbed males still facing sperm competition  
293 at high population bib frequency. We also expect bibbed males to have fewer resources for investing  
294 in ejaculate quality (Kotiaho, 2001, Blount, 2004). The bib is likely carotenoid based, as male painted

295 dragons are known to use carotenoids to produce yellow head colouration (Olsson et al., 2008,  
296 Olsson et al., 2012), and carotenoid-pigment is widely considered costly due to environmental  
297 availability and investment trade-offs (e.g. antioxidant activity or immune function; Olson & Owens,  
298 1998, Svensson & Wong, 2011). Instead of sperm performance traits, the energetic allocation trade-  
299 off with bib expression may be with investment into other life-history traits such as somatic  
300 investment, reactive oxygen production, antioxidant capacity or growth rate between bib-morphs  
301 (e.g. Thompson et al., 1993, Dijkstra et al., 2016). Frequent aggressive activity by bibbed males may  
302 also provide a mechanistic link to these, as aggression and territorial patrolling behaviour may  
303 increase energetic costs, stress and testosterone-mediated immunosuppression, and oxidative stress  
304 (Folstad & Karter, 1992, Whiting et al., 2003, Briffa & Sneddon, 2007, Clark, 2012, Sharick et al., 2015,  
305 Costantini, 2014). Previous data supports this possibility as bibbed males lose body condition at a  
306 greater rate than non-bibbed males when under physiological or social stress in the wild (Olsson et  
307 al., 2009a, Healey & Olsson, 2009).

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

321 energetic trade-off between pre- and postcopulatory traits: that a certain energy allocation is made  
322 to reproductive traits and then subdivided between pre- and postcopulatory traits.

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

### 338 **Sperm performance**

339 Sperm velocity and percentage of motile sperm are important postcopulatory traits across a number  
340 of internally fertilising taxa (Møller, 1988, Firman & Simmons, 2010, Simmons & Fitzpatrick, 2012),  
341 however to our knowledge this has not been tested in a reptile species, so it is possible that other  
342 postcopulatory traits, such as longevity, are more influential for fertilisation success. Female *C. pictus*  
343 can store sperm from multiple males in their reproductive tract for extended periods of time (Olsson  
344 et al., 2009b). There is therefore likely a selective advantage for sperm longevity (Dziminski et al.,  
345 2009, Fitzpatrick et al., 2009), which may even be inversely related to sperm velocity (Levitan, 2000,



346 Helfenstein et al., 2010b, Smith, 2012). Other traits that may be of greater importance for  
347 postcopulatory competition between males include sperm number per ejaculate (Gage & Barnard,  
348 1996, Gage & Morrow, 2003, Boschetto et al., 2011), sperm morphology (Miller & Pitnick, 2002,  
349 Bakker et al., 2014, Kahrl & Cox, 2015), non-sperm ejaculate components (Poiani, 2006, Simmons &  
350 Fitzpatrick, 2012, Crean et al., 2016), or females biasing fertilisation success (cryptic female choice;  
351 Eberhard, 1996, Pitnick et al., 2009). These factors remain to be explored.

## 352 **Conclusion**

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

359

## 360 **Acknowledgements**

361 We have no competing interests. We thank the Australian Research Council (MO), the National  
362 Science Foundation (CF), and The University of Sydney (MO, CM) for financial support, Ernie Snaith  
363 for logistic support and Adele Haythornthwaite for administrative support. Animals were collected  
364 under a permit issued by NSW National Parks and Wildlife Service (SL100352) and this study was  
365 conducted in accordance with University of Sydney ethics approval (AEC-2013/6050).

## 366 **Figure Legends**

367

368 **Figure 1.** Diagrammatic representation of the arenas during the three stages of each female  
369 choice trial, with two condition- and colour-morph matched males, one with a bib (B) and one  
370 with no bib (NB) randomly assigned a side, and a receptive female. A) Acclimation period,  
371 visibility between all lizards is blocked. B) Female visually exposed to the two males. C) Female

372 can approach males. Trial finishes if female makes a 'choice', defined as her head contacting the  
373 transparent screen in front of a male's compartment. Trials are then performed twice more, with  
374 males switching sides each trial. Female 'prefers' a male she chooses at least two times.

375 **Figure 2.** Diagrammatic representation of the arena in which the staged contest trials were  
376 performed. After a 3-minute acclimation period male compartments were removed, exposing  
377 the males to each other directly, and to the receptive female through the mesh.  
378

379 **Figure 3.** Number of times the receptive female preferred (chose in at least two of three  
380 consecutive trials) bibbed and non-bibbed males. The number of staged contests won by bibbed  
381 and non-bibbed males.  
382

## 383 References

- 384 Allen, B. J. & Levinton, J. S. 2007. Costs of bearing a sexually selected ornamental weapon in a fiddler  
385 crab. *Funct. Ecol.* **21**: 154-161.
- 386 Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.
- 387 Andersson, M. & Simmons, L. W. 2006. Sexual selection and mate choice. *Trends Ecol. Evol.* **21**: 296-  
388 302.
- 389 Bakker, T. C., Hollmann, M., Mehlis, M. & Zbinden, M. 2014. Functional variation of sperm  
390 morphology in sticklebacks. *Behav. Ecol. Sociobiol.* **68**: 617-627.
- 391 Barlow, G. W. 1973. COMPETITION BETWEEN COLOR MORPHS OF POLYCHROMATIC MIDAS CICHLID  
392 CICHLASOMA CITRINELLUM. *Science* **179**: 806-807.
- 393 Blount, J. D. 2004. Carotenoids and life-history evolution in animals. *Arch. Biochem. Biophys.* **430**: 10-  
394 15.
- 395 Boschetto, C., Gasparini, C. & Pilastro, A. 2011. Sperm number and velocity affect sperm competition  
396 success in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* **65**: 813-821.
- 397 Briffa, M. & Sneddon, L. U. 2007. Physiological constraints on contest behaviour. *Funct. Ecol.* **21**: 627-  
398 637.
- 399 Clark, C. J. 2012. The role of power versus energy in courtship: what is the 'energetic cost' of a  
400 courtship display? *Anim. Behav.* **84**: 269-277.
- 401 Cogger, H. 2014. *Reptiles and amphibians of Australia*. CSIRO Publishing, Collingwood.
- 402 Costantini, D. 2014. *Oxidative stress and hormesis in evolutionary ecology and physiology. A marriage*  
403 *between mechanistic and evolutionary approaches*. Springer, Berlin.
- 404 Cox, R. M., Zilberman, V. & John-Alder, H. B. 2008. Testosterone stimulates the expression of a social  
405 color signal in Yarrow's spiny lizard, *Sceloporus jarrovii*. *J. Exp. Biol.* **209A**: 505-514.
- 406 Crean, A. J., Adler, M. I. & Bonduriansky, R. 2016. Seminal fluid and mate choice: new predictions.  
407 *Trends Ecol. Evol.* **31**: 253-255.
- 408 Cuervo, J. J. & Shine, R. 2007. Hues of a dragon's belly: morphological correlates of ventral coloration  
409 in water dragons. *J. Zool.* **273**: 298-304.
- 410 Devigili, A., Evans, J. P., Di Nisio, A. & Pilastro, A. 2015. Multivariate selection drives concordant  
411 patterns of pre- and postcopulatory sexual selection in a livebearing fish. *Nat. Commun.* **6**: 9.
- 412 Dewsbury, D. A. 1982. EJACULATE COST AND MALE CHOICE. *American Naturalist* **119**: 601-610.
- 413 Dijkstra, P. D., Pierotti, M. E., Seehausen, O. & Metcalfe, N. B. 2016. Metabolism, oxidative stress and  
414 territorial behaviour in a female colour polymorphic cichlid fish. *Behavioral Ecology and*  
415 *Sociobiology* **70**: 99-109.
- 416 Dines, J. P., Mesnick, S. L., Ralls, K., May-Collado, L., Agnarsson, I. & Dean, M. D. 2015. A trade-off  
417 between precopulatory and postcopulatory trait investment in male cetaceans. *Evolution* **69**:  
418 1560-1572.

419 Dunn, J. C., Halenar, L. B., Davies, T. G., Cristobal-Azkarate, J., Reby, D., Sykes, D., Degg, S., Fitch, W.  
420 T. & Knapp, L. A. 2015. Evolutionary Trade-Off between Vocal Tract and Testes Dimensions in  
421 Howler Monkeys. *Curr. Biol.* **25**: 2839-2844.

422 Dziminski, M. A., Roberts, J. D., Beveridge, M. & Simmons, L. W. 2009. Sperm competitiveness in  
423 frogs: slow and steady wins the race. *Proc. R. Soc. B-Biol. Sci.* **276**: 3955-3961.

424 Eberhard, W. G. 1996. *Female control: sexual selection by cryptic female choice*. Princeton University  
425 Press, Princeton.

426 Emlen, D. J. 2001. Costs and the diversification of exaggerated animal structures. *Science* **291**: 1534-  
427 1536.

428 Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and the evolution of mating systems.  
429 *Science* **197**: 215-23.

430 Evans, J. P. & Garcia-Gonzalez, F. 2016. The total opportunity for sexual selection and the integration  
431 of pre-and post-mating episodes of sexual selection in a complex world. *J. Evol. Biol.*

432 Evans, J. P., Zane, L., Francescato, S. & Pilastro, A. 2003. Directional postcopulatory sexual selection  
433 revealed by artificial insemination. *Nature* **421**: 360-363.

434 Firman, R. C. & Simmons, L. W. 2010. Experimental evolution of sperm quality via postcopulatory  
435 sexual selection in house mice. *Evolution* **64**: 1245-1256.

436 Fitzpatrick, J., Desjardins, J., Milligan, N., Montgomerie, R. & Balshine, S. 2007. Reproductive-tactic-  
437 specific variation in sperm swimming speeds in a shell-brooding cichlid. *Biol. Reprod.* **77**: 280-  
438 284.

439 Fitzpatrick, J. L., Almbro, M., Gonzalez-Voyer, A., Kolm, N. & Simmons, L. W. 2012. Male contest  
440 competition and the coevolution of weaponry and testes in pinnipeds. *Evolution* **66**: 3595-  
441 3604.

442 Fitzpatrick, J. L. & Lupold, S. 2014. Sexual selection and the evolution of sperm quality. *Molecular*  
443 *Human Reproduction* **20**: 1180-1189.

444 Fitzpatrick, J. L., Montgomerie, R., Desjardins, J. K., Stiver, K. A., Kolm, N. & Balshine, S. 2009. Female  
445 promiscuity promotes the evolution of faster sperm in cichlid fishes. *Proceedings of the*  
446 *National Academy of Sciences* **106**: 1128-1132.

447 Fleishman, L. J. 1992. The influence of the sensory system and the environment on motion patterns  
448 in the visual-displays of anoline lizards and other vertebrates. *Am. Nat.* **139**: S36-S61.

449 Folstad, I. & Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am.*  
450 *Nat.* **139**: 603-622.

451 Friesen, C. R., Powers, D. R., Copenhaver, P. E. & Mason, R. T. 2015. Size dependence in non-sperm  
452 ejaculate production is reflected in daily energy expenditure and resting metabolic rate.  
453 *Journal of Experimental Biology* **218**: 1410-1418.

454 Friesen, C. R., Shine, R., Krohmer, R. W. & Mason, R. T. 2013. Not just a chastity belt: the functional  
455 significance of mating plugs in garter snakes, revisited. *Biological Journal of the Linnean*  
456 *Society* **109**: 893-907.

457 Friesen, C. R., Squire, M. K. & Mason, R. T. 2014. Intrapopulational variation of ejaculate traits and  
458 sperm depletion in red-sided garter snakes. *J. Zool.* **292**: 192-201.

459 Friesen, C. R., Wilson, M. R., Rollings, N., Sudyka, J., Whittington, C. M., Giraudeau, M. & Olsson, M.  
460 2017. Conditional handicaps in exuberant lizards: bright color in aggressive males is  
461 correlated with high levels of free radicals. *Front. Ecol. Evol.* **5**: 1-9.

462 Gage, A. & Barnard, C. 1996. Male crickets increase sperm number in relation to competition and  
463 female size. *Behav. Ecol. Sociobiol.* **38**: 349-353.

464 Gage, M. J. & Morrow, E. H. 2003. Experimental evidence for the evolution of numerous, tiny sperm  
465 via sperm competition. *Curr. Biol.* **13**: 754-757.

466 Giraudeau, M., Friesen, C. R., Sudyka, J., Rollings, N., Whittington, C. M., Wilson, M. R. & Olsson, M.  
467 2016. Ageing and the cost of maintaining coloration in the Australian painted dragon. *Biology*  
468 *Letters* **12**: 20160077.

469 Healey, M. & Olsson, M. 2009. Too big for his boots: Are social costs keeping condition-dependent  
470 status signalling honest in an Australian lizard? *Austral Ecology* **34**: 636-640.

- 471 Healey, M., Uller, T. & Olsson, M. 2007. Seeing red: morph-specific contest success and survival rates  
472 in a colour-polymorphic agamid lizard. *Animal Behaviour* **74**: 337-341.
- 473 Healey, M., Uller, T. & Olsson, M. 2008. Variety is the spice of life: female lizards choose to associate  
474 with colour-polymorphic male dyads. *Ethology* **114**: 231-237.
- 475 Helfenstein, F., Losdat, S., Møller, A. P., Blount, J. D. & Richner, H. 2010a. Sperm of colourful males  
476 are better protected against oxidative stress. *Ecol. Lett.* **13**: 213-222.
- 477 Helfenstein, F., Podelvin, M. & Richner, H. 2010b. Sperm morphology, swimming velocity, and  
478 longevity in the house sparrow *Passer domesticus*. *Behavioral Ecology and Sociobiology* **64**:  
479 557-565.
- 480 Hosken, D. J., Taylor, M. L., Hoyle, K., Higgins, S. & Wedell, N. 2008. Attractive males have greater  
481 success in sperm competition. *Curr. Biol.* **18**: R553-R554.
- 482 Hsu, Y. Y., Earley, R. L. & Wolf, L. L. 2006. Modulation of aggressive behaviour by fighting experience:  
483 mechanisms and contest outcomes. *Biol. Rev. Camb. Philos. Soc.* **81**: 33-74.
- 484 Isaksson, C., Uller, T. & Andersson, S. 2006. Parental effects on carotenoid-based plumage coloration  
485 in nestling great tits, *Parus major*. *Behav. Ecol. Sociobiol.* **60**: 556-562.
- 486 Kahrl, A. F., Cox, C. L. & Cox, R. M. 2016. Correlated evolution between targets of pre-and  
487 postcopulatory sexual selection across squamate reptiles. *Ecol. Evol.* **6**: 6452-6459.
- 488 Kahrl, A. F. & Cox, R. M. 2015. Diet affects ejaculate traits in a lizard with condition-dependent  
489 fertilization success. *Behav. Ecol.*: 1502-1511.
- 490 Kemp, D. J., Herberstein, M. E., Fleishman, L. J., Endler, J. A., Bennett, A. T., Dyer, A. G., Hart, N. S.,  
491 Marshall, J. & Whiting, M. J. 2015. An integrative framework for the appraisal of coloration in  
492 nature. *Am. Nat.* **185**: 705-724.
- 493 Kempnaers, B., Verheyen, G. R., Vandenbroeck, M., Burke, T., Van Broeckhoven, C. & Dhondt, A. A.  
494 1992. Extra-pair paternity results from female preference for high-quality males in the blue  
495 tit. *Nature* **357**: 494-496.
- 496 Kotiaho, J. S. 2001. Costs of sexual traits: a mismatch between theoretical considerations and  
497 empirical evidence. *Biol. Rev. Camb. Philos. Soc.* **76**: 365-376.
- 498 Kvarnemo, C. & Simmons, L. W. 2013. Polyandry as a mediator of sexual selection before and after  
499 mating. *Philos. Trans. R. Soc B* **368**: 1-8.
- 500 Lancaster, L. T., Hipsley, C. A. & Sinervo, B. 2009. Female choice for optimal combinations of multiple  
501 male display traits increases offspring survival. *Behav. Ecol.* **20**: 993-999.
- 502 Lattanzio, M. S., Metro, K. J. & Miles, D. B. 2014. Preference for male traits differ in two female  
503 morphs of the tree lizard, *Urosaurus ornatus*. *PLOS ONE* **9**: 1-9.
- 504 Levitan, D. R. 2000. Sperm velocity and longevity trade off each other and influence fertilization in  
505 the sea urchin *Lytechinus variegatus*. *Proc. Biol. Sci.* **267**: 531-534.
- 506 Lewis, Z., Lize, A. & Wedell, N. 2013. The interplay between different stages of reproduction in males  
507 of the moth *Plodia interpunctella*. *Anim. Behav.* **86**: 917-922.
- 508 Locatello, L., Rasotto, M. B., Evans, J. P. & Pilastro, A. 2006. Colourful male guppies produce faster  
509 and more viable sperm. *J. Evol. Biol.* **19**: 1595-1602.
- 510 Lupold, S., Tomkins, J. L., Simmons, L. W. & Fitzpatrick, J. L. 2014. Female monopolization mediates  
511 the relationship between pre- and postcopulatory sexual traits. *Nat. Commun.* **5**: 1-8.
- 512 Malo, A. F., Roldan, E. R. S., Garde, J., Soler, A. J. & Gomendio, M. 2005. Antlers honestly advertise  
513 sperm production and quality. *Proc. Biol. Sci.* **272**: 149-157.
- 514 Marie-Orleach, L., Janicke, T., Vizoso, D. B., David, P. & Scharer, L. 2016. Quantifying episodes of  
515 sexual selection: Insights from a transparent worm with fluorescent sperm. *Evolution* **70**:  
516 314-328.
- 517 Mattson, K. J., De Vries, A., McGuire, S. M., Krebs, J., Louis, E. E. & Loskutoff, N. M. 2007. Successful  
518 artificial insemination in the corn snake, *Elaphe gutatta*, using fresh and cooled semen. *Zoo*  
519 *Biol.* **26**: 363-369.
- 520 McKinnon, J. S. & Pierotti, M. E. R. 2010. Colour polymorphism and correlated characters: genetic  
521 mechanisms and evolution. *Molecular Ecology* **19**: 5101-5125.
- 522 Miller, G. T. & Pitnick, S. 2002. Sperm-female coevolution in *Drosophila*. *Science* **298**: 1230-3.

- 523 Møller, A. P. 1988. Ejaculate quality, testes size and sperm competition in primates. *J. Hum. Evol.* **17**:  
524 479-488.
- 525 Naretto, S., Blengini, C. S., Cardozo, G. & Chiaraviglio, M. 2016. Pre- and Postcopulatory Traits of  
526 Salvator Male Lizards in Allopatry and Sympatry. *Scientifica*: 9.
- 527 Olson, V. A. & Owens, I. P. F. 1998. Costly sexual signals: are carotenoids rare, risky or required?  
528 *Trends in Ecology & Evolution* **13**: 510-514.
- 529 Olsson, M., Healey, M. & Astheimer, L. 2007a. Afternoon T: Testosterone level is higher in red than  
530 yellow male polychromatic lizards. *Physiol. Behav.* **91**: 531-534.
- 531 Olsson, M., Healey, M., Wapstra, E., Schwartz, T., Lebas, N. & Uller, T. 2007b. Mating system variation  
532 and morph fluctuations in a polymorphic lizard. *Mol. Ecol.* **16**: 5307-5315.
- 533 Olsson, M., Healey, M., Wapstra, E. & Uller, T. 2009a. Testing the quality of a carrier: a field  
534 experiment on lizard signalers. *Evolution* **63**: 695-701.
- 535 Olsson, M. & Madsen, T. 1995. Female choice on male quantitative traits in lizards - why is it so rare?  
536 *Behav. Ecol. Sociobiol.* **36**: 179-184.
- 537 Olsson, M. & Madsen, T. (1998) Sexual selection and sperm competition in reptiles. In: *Sperm*  
538 *Competition and Sexual Selection*, (Birkhead, T. R. & Møller, A. P., eds.). pp. 503-578.  
539 Academic Press, San Diego.
- 540 Olsson, M., Madsen, T. & Shine, R. 1997. Is sperm really so cheap? Costs of reproduction in male  
541 adders, *Vipera berus*. *Proc. Biol. Sci.* **264**: 455-459.
- 542 Olsson, M., Schwartz, T., Uller, T. & Healey, M. 2009b. Effects of sperm storage and male colour on  
543 probability of paternity in a polychromatic lizard. *Anim. Behav.* **77**: 419-424.
- 544 Olsson, M., Stuart-Fox, D. & Ballen, C. 2013. Genetics and evolution of colour patterns in reptiles.  
545 *Semin. Cell Dev. Biol.* **24**: 529-541.
- 546 Olsson, M., Tobler, M., Healey, M., Perrin, C. & Wilson, M. 2012. A significant component of ageing  
547 (DNA damage) is reflected in fading breeding colors: an experimental test using innate  
548 antioxidant mimetics in painted dragon lizards. *Evolution* **66**: 2475-2483.
- 549 Olsson, M., Ujvari, B., Wapstra, E., Madsen, T., Shine, R. & Bensch, S. 2005. Does mate guarding  
550 prevent rival mating in snow skinks? A test using AFLP. *Herpetologica* **61**: 389-394.
- 551 Olsson, M., Wilson, M., Isaksson, C., Uller, T. & Mott, B. 2008. Carotenoid intake does not mediate a  
552 relationship between reactive oxygen species and bright colouration: experimental test in a  
553 lizard. *Journal of Experimental Biology* **211**: 1257-1261.
- 554 Oyegbile, T. O. & Marler, C. A. 2005. Winning fights elevates testosterone levels in California mice  
555 and enhances future ability to win fights. *Hormones and Behavior* **48**: 259-267.
- 556 Parker, G. A. 1970. SPERM COMPETITION AND ITS EVOLUTIONARY CONSEQUENCES IN INSECTS.  
557 *Biological Reviews of the Cambridge Philosophical Society* **45**: 525-&.
- 558 Parker, G. A., Lessells, C. M. & Simmons, L. W. 2013. Sperm competition games: a general model for  
559 precopulatory male-male competition. *Evolution* **67**: 95-109.
- 560 Parker, G. A. & Pizzari, T. 2010. Sperm competition and ejaculate economics. *Biological Reviews* **85**:  
561 897-934.
- 562 Pilastro, A., Simonato, M., Bisazza, A. & Evans, J. P. 2004. Cryptic female preference for colorful males  
563 in guppies. *Evolution* **58**: 665-669.
- 564 Pischedda, A. & Rice, W. R. 2012. Partitioning sexual selection into its mating success and fertilization  
565 success components. *Proceedings of the National Academy of Sciences of the United States*  
566 *of America* **109**: 2049-2053.
- 567 Pitcher, T. E., Rodd, F. & Rowe, L. 2007. Sexual colouration and sperm traits in guppies. *J. Fish Biol.*  
568 **70**: 165-177.
- 569 Pitnick, S., Miller, G. T., Reagan, J. & Holland, B. 2001. Males' evolutionary responses to experimental  
570 removal of sexual selection. *Proc. R. Soc. B-Biol. Sci.* **268**: 1071-1080.
- 571 Pitnick, S., Wolfner, M. F. & Suarez, S. S. (2009) Ejaculate-female and sperm-female interactions. In:  
572 *Sperm Biology: An Evolutionary Perspective*, (Birkhead, T. R., Hosken, D. J. & Pitnick, S., eds.).  
573 pp. 247-304. Elsevier/Academic Press, London.
- 574 Poiani, A. 2006. Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* **60**: 289-310.

575 Pryke, S. R., Andersson, S. & Lawes, M. J. 2001. Sexual selection of multiple handicaps in the red-  
576 collared widowbird: female choice of tail length but not carotenoid display. *Evolution* **55**:  
577 1452-1463.

578 Pryke, S. R., Astheimer, L. B., Buttemer, W. A. & Griffith, S. C. 2007. Frequency-dependent  
579 physiological trade-offs between competing colour morphs. *Biol. Lett.* **3**: 494-497.

580 Ratti, O., Hovi, M., Lundberg, A., Tegelstrom, H. & Alatalo, R. V. 1995. Extra-pair paternity and male  
581 characteristics in the pied flycatcher. *Behav. Ecol. Sociobiol.* **37**: 419-425.

582 Rowe, M., Swaddle, J. P., Pruett-Jones, S. & Webster, M. S. 2010. Plumage coloration, ejaculate  
583 quality and reproductive phenotype in the red-backed fairy-wren. *Anim. Behav.* **79**: 1239-  
584 1246.

585 Schulte-Hostedde, A. I., Zinner, B., Millar, J. S. & Hickling, G. J. 2005. Restitution of mass-size  
586 residuals: Validating body condition indices. *Ecology* **86**: 155-163.

587 Sharick, J. T., Vazquez-Medina, J. P., Ortiz, R. M. & Crocker, D. E. 2015. Oxidative stress is a potential  
588 cost of breeding in male and female northern elephant seals. *Funct. Ecol.* **29**: 367-376.

589 Shuster, S. M. & Wade, M. J. (2003) Mating systems and strategies. In: *Monographs in behavior and*  
590 *ecology*. pp. Princeton University Press, Princeton, New Jersey.

591 Simmons, L. W. & Fitzpatrick, J. L. 2012. Sperm wars and the evolution of male fertility. *Reproduction*  
592 **144**: 519-534.

593 Smith, C. C. 2012. Opposing effects of sperm viability and velocity on the outcome of sperm  
594 competition. *Behavioral Ecology* **23**: 820-826.

595 Svensson, P. A. & Wong, B. B. M. 2011. Carotenoid-based signals in behavioural ecology: a review.  
596 *Behaviour* **148**: 131-189.

597 Taborsky, M. 1998. Sperm competition in fish: 'bourgeois' males and parasitic spawning. *Trends in*  
598 *Ecology & Evolution* **13**: 222-227.

599 Team, R. D. C. (2010) R: a language and environment for statistical computing. In: *R Foundation for*  
600 *Statistical Computing*, Vol. <https://cran.r-project.org/>. pp. R Foundation for Statistical  
601 Computing, Vienna, Austria.

602 Thompson, C., Moore, I. & Moore, M. 1993. Social, environmental and genetic factors in the  
603 ontogeny of phenotypic differentiation in a lizard with alternative male reproductive  
604 strategies. *Behav. Ecol. Sociobiol.* **33**: 137-146.

605 Tokarz, R. R. 1995. Mate choice in lizards: a review. *Herpetol. Monogr.* **9**: 17-40.

606 Travers, L. M., Garcia-Gonzalez, F. & Simmons, L. W. 2016. Genetic variation but weak genetic  
607 covariation between pre- and post-copulatory episodes of sexual selection in *Drosophila*  
608 *melanogaster*. *Journal of Evolutionary Biology* **29**: 1535-1552.

609 Tuttle, E. M. 2003. Alternative reproductive strategies in the white-throated sparrow: behavioral and  
610 genetic evidence. *Behav. Ecol.* **14**: 425-432.

611 Uller, T. & Olsson, M. 2008. Multiple paternity in reptiles: patterns and processes. *Mol. Ecol.* **17**:  
612 2566-2580.

613 Vercken, E., Massot, M., Sinervo, B. & Clobert, J. 2007. Colour variation and alternative reproductive  
614 strategies in females of the common lizard *Lacerta vivipara*. *J. Evol. Biol.* **20**: 221-232.

615 Whiting, M. J., Nagy, K. A. & Bateman, P. W. (2003) Evolution and maintenance of social status  
616 signalling badges: experimental manipulations in lizards. In: *Lizard Social Behavior*, (Fox, S. F.,  
617 McCoy, J. K. & Baird, T. A., eds.). pp. Johns Hopkins University Press, Baltimore (MD).

618 Yewers, M. S. C., Pryke, S. & Stuart-Fox, D. 2016. Behavioural differences across contexts may  
619 indicate morph-specific strategies in the lizard *Ctenophorus decresii*. *Anim. Behav.* **111**: 329-  
620 339.

621