

Why are females ornamented? A test of the courtship stimulation and courtship rejection hypotheses

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Female ornamentation was initially thought to reflect genetic correlation with the more elaborate male trait. However, this cannot explain female-specific ornamentation, such as the conspicuous coloration displayed by females of many species during breeding. Females may exhibit distinctive, reproductive coloration to 1) advertise receptivity and stimulate male courtship or 2) advertise nonreceptivity when gravid to reduce male courtship, harassment, and potentially costly copulations. We tested both hypotheses in the Lake Eyre dragon lizard (*Ctenophorus maculosus*) by quantifying female coloration, using spectroradiometry and a model of lizard color perception, and male and female behavior across the female reproductive cycle. Females develop bright orange coloration on their throat and abdomen during the breeding season, whereas males remain cryptically colored. The onset of orange coloration was associated with enlarging follicles, acceptance of copulations, and escalation of male courtship. Rather than fading once females were no longer receptive, however, the intense orange coloration remained until oviposition. Furthermore, despite maximal coloration associated with nonreceptivity, males persisted with courtship and copulation attempts, and females increased rejection behaviors comprising lateral displays and flipping onto their backs (to prevent forced intromission), both of which emphasize the conspicuous ventrolateral coloration. These apparently costly rejection behaviors did not reduce male harassment but did decrease the frequency of potentially costly copulations. These results suggest that 1) males do not determine female receptivity based on coloration alone and 2) the potentially costly rejection behaviors may have evolved to reduce the direct costs of mating. *Key words:* color perception, cost of mating, female resistance, lizard, reproduction, sexual conflict, visual modeling. [*Behav Ecol* 20:1334–1342 (2009)]

Evolutionary understanding of conspicuous coloration and ornamentation has focused primarily on males because in most species males are the more elaborately ornamented sex. Female ornamentation was largely overlooked because it was thought to reflect genetic correlation with the male trait (Lande 1980; Amundsen 2000a, 2000b). However, there is increasing recognition that female ornamentation may be under direct selection. Evidence for direct selection on female ornamentation comes from both experimental studies of sexual selection acting directly on females (reviewed in Amundsen 2000a, 2000b; Kraaijeveld et al. 2004) and phylogenetic comparative studies showing that female ornamentation often evolves independently from male ornamentation (Irwin 1994; Burns 1998). In most species, female ornamentation resembles that of males, though it is often much reduced, and hence, the roles of genetic correlation and direct selection may be difficult to disentangle. Species where only the females are ornamented, however, provide an ideal opportunity to examine direct selection on the female trait.

Most studies of female-specific ornamentation have focussed on sex-role reversed or mutually ornamented species, where females compete for mates and males provide a large proportion of parental investment (e.g., Rowland et al. 1991; Roulin et al. 2001; Kraaijeveld et al. 2004; Torres and Velando 2005). In these systems, female-specific ornamentation can generally be explained by sexual selection via female–female competi-

tion and/or male mate choice. However, many examples of female-specific ornamentation are found in species with conventional sex-roles (e.g., Montgomerie and Thornhill 1989; Cooper and Greenberg 1992; Watkins 1997; Nunn 1999; Cuadrado 2000; Amundsen and Forsgren 2001; Baird 2004; Heinsohn et al. 2005; Weiss 2006). In the latter, female ornamentation is often expressed seasonally or periodically and often signals some aspect of female reproductive status, although it may simultaneously signal other aspects of female quality (Amundsen and Forsgren 2001). In addition to facilitating recognition of sex and reproductive maturity, such conspicuous coloration may have evolved for 2 main reasons associated with reproductive status. First, bright coloration may allow females to publicize the most effective time for fertilization and function to stimulate male courtship or incite male–male competition (reviewed in Cooper and Greenberg 1992; Nunn 1999). The courtship stimulation hypothesis predicts that females should exhibit bright coloration when receptive and that female coloration should increase male courtship (Cooper and Greenberg 1992). Second, female coloration may advertise nonreceptivity in order to decrease the direct costs of male harassment and mating (Cooper and Greenberg 1992). The courtship rejection hypothesis predicts that bright coloration should develop when females are unreceptive and should decrease male courtship, harassment, and copulation attempts (Cooper and Greenberg 1992). Alternatively, female coloration may constitute a conditional signal, varying in intensity in relation to reproductive status and behavior. For instance, low-intensity coloration may signal receptivity, and higher intensity coloration, coupled with behavioral rejection, may signal nonreceptivity (Cooper and Greenberg 1992).

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Received 4 September 2008; revised 29 June 2009; accepted 19 July 2009.

Lizards are an ideal group in which to study the role of female ornamentation in signaling reproductive status because they have conventional sex-roles, promiscuous mating systems, and no parental care and females of many species develop conspicuous coloration during the breeding season (reviewed in Cooper and Greenberg 1992). In lizards, female-specific coloration has been shown to play a role in both courtship stimulation (Cuadrado 2000; Weiss 2002; Baird 2004) and rejection (Cuadrado 2000; Weiss 2002; Stuart-Fox and Whiting 2005) or both (Cuadrado 2000; Hager 2001; Weiss 2002). However, detailed experimental studies quantifying female color expression independently of the human visual system, in conjunction with male and female reproductive behavior, are currently lacking. Here, we test the courtship stimulation and rejection hypotheses in the Australian Lake Eyre dragon lizard, *Ctenophorus maculosus*. We quantified both female color expression using spectroradiometry and a model of lizard color perception and female behavior throughout the reproductive cycle. We also experimentally tested whether the coloration and reproductive behavior of females affected male courtship and harassment behavior.

Lake Eyre dragons are an especially novel system in which to test these hypotheses as females develop extensive, conspicuous, ventrolateral orange coloration (Supplementary Figure S1) during the breeding season, whereas unusually, males remain cryptically colored (Mitchell 1973). The species relies almost exclusively on visual signals and has not been observed to tongue flick (to sample chemical cues) due to its extremely arid and saline habitat (Mitchell 1973, Devi Stuart-Fox DS-F, personal observation). Mating is potentially costly for females as they can be injured or killed by the male's mating grasp, and males often attempt to force copulations (Olsson 1995a). In response to forced copulation attempts, female *C. maculosus* has evolved a unique sequence of rejection behaviors. Initially, females either flee or perform lateral displays where they elevate themselves on all 4 legs, laterally compress the body, and arch the back, revealing their ventrolateral orange coloration. However, as a last resort, females flip themselves onto their backs exposing bright ventrolateral orange coloration because this position prevents forced male intromission (Olsson 1995a). This behavior has not been reported in any other lizard. Given that the species only occurs on the barren salt pans of Australia's arid interior, this behavior seems particularly risky as the bright orange and white ventral coloration is highly conspicuous against the brownish salt crust (Olsson 1995a). *Ctenophorus maculosus* exhibit highly cryptic dorsal coloration and rely on crypsis (crouching on the salt crust rather than fleeing into burrows) for defense against their predators, which are primarily birds and sand goannas, *Varanus gouldii* (Mitchell 1973; Olsson 1995a). As the flipping over behavior may incur a high predation cost to females, Lake Eyre dragons have been widely cited as a vertebrate example of sexual conflict driving the evolution of costly female resistance (e.g., Birkhead 1995; Gavrillets et al. 2001; Eberhard 2002; Cunningham 2003; Shine et al. 2003; Shine and Mason 2005). It is therefore of particular interest to determine how female color expression relates to reproductive behavior and whether such coloration serves a rejection function to reduce costly male harassment and copulation in this system.

MATERIALS AND METHODS

Study system, animal capture, and husbandry

Lake Eyre dragons are small lizards (adult 50–70 mm snout-vent length, SVL) restricted to the large, dry salt lakes in the southern interior of Australia (Lake Eyre, Lake Frome,

and Lake Torrens). They have a number of specialized morphological and physiological adaptations for existence under these extremely arid, hot, and saline conditions (Mitchell 1973). There is no vegetation, and lizards survive on one species of ant, *Melophorus* sp., and opportunistic capture of live or dead insects that have strayed or blown onto the salt crust. Males compete for access to territories and females. Males emerge from mid August to early September and establish territories to defend reproductive rather than food resources, whereas females emerge 2–5 weeks later and have overlapping home ranges within male territories (Olsson 1995b). Lizards spend up to 8 h per day foraging, during which they may travel long distances from their territories (Mitchell 1973). Consequently, females are likely to encounter unfamiliar territorial and nonterritorial “floater” males.

We caught 57 adult lizards (25 females and 32 males) by hand from Lake Eyre, South Australia (28.95–29.05°S, 137.65–137.76°E), between 22 August and 7 September 2007 and maintained them in captivity at the University of Melbourne. The animals were captured shortly after emergence from hibernation, and none of the females showed orange coloration until between 21 and 30 days after being brought into captivity. Body size (SVL) to the nearest millimeter and mass to the nearest 0.1 g were measured at capture and 1 month after being brought into captivity. We calculated body condition as mass/SVL³. The mean (\pm standard deviation [SD]) size of females in this study was 56.6 \pm 4 mm (range 49–64 mm) and of males was 65.2 \pm 3.5 mm (range 56–70 mm).

Lizards were housed individually in 61 \times 30 \times 30 cm glass tanks (separated by opaque partitions) each with a heat lamp and a layer of sand and salt crust to mimic natural habitat. The room was maintained at an average temperature of 28 °C on a 12:12 day:night cycle. Lizards were misted and fed crickets (*Anchieta domesticus*) or mealworms (*Tenbrio* spp. larvae) dusted in calcium and multivitamins 3 times a week.

Female reproductive stages

To quantify reproductive behaviors in relation to color expression, we divided the reproductive cycle into 9 stages (Table 1). These stages were defined to enable us to detect fine-scale

Table 1
Stages defining female color and associated reproductive state in Lake Eyre dragons

Stage	Definition	Coloration	Inferred reproductive status
0	Prior to first d.o.	White	Nonreproductive
1	0–4 days after d.o.	Orange	Vitellogenic follicles not always detectable via palpation
2	5–8 days after d.o.	Orange	Vitellogenic follicles
3	9–12 days after d.o.	Orange	Ovulation most likely around this time
4	13–16 days after d.o.	Orange	Shelling oviductal eggs by the end of stage 4 (latest)
5	17–20 days after d.o.	Orange	Shelling oviductal eggs
6	21 days after d.o. to	Orange	Shelled oviductal eggs laying
7	0–10 days after laying	Faded orange/white	Postoviposition
8	10+ days after laying without orange reintensifying	Faded orange/white	Postreproductive

d.o., developing orange.

temporal changes in color intensity and behavior across the reproductive cycle because precise reproductive status (e.g., timing of ovulation) cannot be determined noninvasively in this species. Females were visually checked daily for appearance of orange coloration, which began as a faint orange wash on the throat (and could be easily detected without handling). Stage 0 was the period at the beginning of the breeding season, where female coloration was white (Supplementary Figure S1). Some females remained white ($N = 7$) associated with reproductive inactivity (i.e., no development of orange coloration or oviposition) over the entire breeding season. The remaining 18 females became orange and produced one or more clutches. Females in stage 0 were therefore classed as being “nonreproductive” (Table 1). Stages 1–5 consisted of 4-day increments, from the date females developed orange coloration, and stage 6 extended to laying date (Table 1). Vitellogenic follicles could be detected via palpation during stages 2 and 3, whereas females had shelled oviductal eggs from stages 4 or 5 until laying. During these 6 stages, females retained their orange coloration (Table 1). Stage 7 was defined as the 10 days after laying, and stage 8 was the period greater than 10 days after laying without the female becoming reproductively active again, with coloration in these 2 stages being either a faded orange or white (Table 1). As *C. maculosus* are a multiclutching species, in some females, orange coloration reintensified within 10 days after laying, and they became reproductively active again, returning to stage 1 of the cycle. The majority of females laid only 1 clutch ($N = 10$), but others laid either 2 ($N = 5$) or 3 clutches ($N = 3$). We recorded the number of eggs and clutch mass for each female.

Female coloration

We quantified female coloration using a spectrometer (USB 4000; Ocean Optics, Dunedin, FI) and a PX-2 light source connected to a fiber-optic reflectance probe mounted within a probe holder. Reflectance readings were taken at a constant 45° angle and sampled from a 5-mm area and calibrated using a Spectralon white certified reflectance standard (USRS-99-010; Labsphere Inc, North Sutton, NH). For analysis, we averaged spectral reflectance over each 5-nm interval using a kernel smoothing function, in the range of 300–700 nm, the visual spectrum for most diurnal lizards (Loew et al. 2002). We took measurements from each side of the throat, the gular stripe, and white chest and 2 measurements from each side of the abdomen (Supplementary Figures S1 and S2a–c). For the throat and abdomen, we used averages of the measurements.

We measured the proportion of orange coloration by taking standardized photographs of the lizards' ventral surface against a background of graph paper. We first imported the images into Adobe Photoshop version 9.0.2 and then traced the area of orange coloration on the throat and abdomen and the total area of the lizard's ventral surface from the cloaca to the tip of the snout (not including limbs). We then calculated the proportion of the lizard's ventral surface occupied by the orange coloration. Proportions were arcsine transformed for statistical analysis.

Visual modeling

To examine changes in female signals across the reproductive cycle, we estimated the visual contrast of the orange and black color patches against the adjacent white ventral surface relative to the lizard visual system. We applied the model of Vorobyev and Osorio (1998), which has been applied to a range of vertebrates (e.g., Siddiqi et al. 2004; Hemmi et al. 2006; Stuart-Fox and Moussalli 2008; and references therein). It assumes

that visual discrimination is limited by photoreceptor noise, ω_i and can be used to estimate the discriminability of 2 colors in units of discrimination thresholds or “just noticeable differences.” We used the same model calculations as detailed in Siddiqi et al. (2004).

Application of this model requires data on irradiance (i.e., illumination) and the visual system of the lizard in addition to the reflectance of lizard colors. We used an irradiance spectrum representing full sunlight (Supplementary Figure S2d) and data on the spectral sensitivities of a congeneric lizard, *Ctenophorus ornatus* (Barbour et al. 2002, Supplementary Figure S2e). As in other related diurnal, visually oriented lizards (chameleons, Loew et al. 2002; iguanids, Bowmaker et al. 2005), this species has a pure cone retina containing both single and double cones. However, Barbour et al. (2002) identified only 3 visual pigment types, a short wavelength-sensitive (SWS; $\lambda_{\max} = 440$ nm), medium wavelength-sensitive (MWS; $\lambda_{\max} = 493$ nm), and long wavelength-sensitive (LWS; $\lambda_{\max} = 571$ nm) type but failed to identify an ultraviolet-sensitive (UVS) photoreceptor that has been found in all other related lizards (e.g., Loew et al. 2002; Bowmaker et al. 2005). This may be because UVS photoreceptors are generally rare, and sample sizes for microspectrophotometric data in Barbour et al. (2002) are small. Thus, we repeated all model calculations with and without a fourth UVS photoreceptor ($\lambda_{\max} = 360$ nm). Results in terms of color variation across the reproductive cycle remained qualitatively unchanged; so, we only present results based on the 3 published photoreceptor spectral sensitivities. Visual pigment absorbance curves were multiplied by the transmission spectra of oil droplets and the ocular media (lens and cornea; assumed to have a $\lambda_{\text{cutoff}} = 350$ nm as in other lizards) and then normalized to equal area (Endler and Mielke 2005). The oil droplets likely to be associated with the SWS, MWS, and LWS cones were transparent, yellow ($\lambda_{\text{cutoff}} = 425$ nm), and yellow-green ($\lambda_{\text{cutoff}} = 520$ nm), respectively (Barbour et al. 2002).

We first derived receptor quantum catches for each cone type over the visible spectrum (Vorobyev and Osorio 1998; Endler and Mielke 2005) and then applied the von Kries transformation to account for receptor adaptation to the light environment, which contributes to color constancy (Vorobyev and Osorio 1998; Vorobyev et al. 1998; Siddiqi et al. 2004; Endler and Mielke 2005). We assumed that photoreceptor noise, ω_i for the LWS photoreceptor = 0.05 and then derived ω_i for remaining photoreceptor classes (see Stuart-Fox et al. 2003; Siddiqi et al. 2004). We used calculations for high-illumination conditions because the habitat is open, and the lizards are diurnal. We used a ratio of 1:3.5:6 for the 3 photoreceptor classes (Barbour et al. 2002). We assumed that as in other vertebrates, the 3 single cones are used for chromatic (color) discrimination, whereas the LWS photoreceptors in the double cones are used for achromatic (brightness) discrimination (Osorio and Vorobyev 2005).

Female and male behavior

We conducted behavioral trials over the lizards' breeding season from September 2007 to January 2008 during the lizards' period of peak activity (0900–1300 h). We placed females in male tanks and videotaped the interaction for approximately 30 min (mean \pm SD = 33.2 \pm 4.4 min) using a Panasonic SDR-H250 digital video camera. Males were initially randomly assigned to females and subsequently chosen so that no female was paired with the same male within a single reproductive cycle. Individuals were only used in one trial per day, on no more than 2 consecutive days, and females were trialed no more than twice during a single breeding stage to minimize stress and use

Table 2
Sample sizes for measurements of female coloration (spectrometry and relative area) and male and female behavior

Female stage	Spectrometry	Relative area of orange	Reproductive behavior
0	14 (13)	0	32 (16)
1	7 (7)	10 (10)	18 (9)
2	10 (8)	10 (10)	19 (10)
3	12 (10)	13 (13)	19 (10)
4	19 (14)	13 (13)	20 (10)
5	7 (7)	11 (11)	20 (10)
6	8 (7)	6 (6)	20 (10)
7	10 (9)	11 (11)	18 (9)
8	11 (11)	8 (8)	20 (10)
Total	98	82	186

In each case, the number of measurements is given with the number of females represented in parenthesis. The same individual was used in multiple stages (the total number of females used in the study was 25).

of individual animals (Table 2). We scored female and male reproductive behaviors from the video footage.

Behavioral interactions generally began with the male courting the female with a series of head bobs, push-ups, and short leaps toward the female (behaviors defined in Supplementary Table S1). In response, females would perform head bobs and arm waves, allow copulation, flee, or reject the male by flipping over or using a lateral display. We scored the number of male and female head bobs, female arm waves, copulation duration, and the duration of lateral displays and flips. When females rejected male courtship and copulation attempts, the male would harass her by chasing and biting in an attempt to gain a mating grasp to force copulation. We scored the duration of male biting as a measure of potentially costly harassment. All behaviors were converted to measures per minute of active trial time, defined as the time from when the individuals first interacted and excluding the time that each lizard spent hiding beneath the salt crust. Behavioral data were log transformed to meet model assumptions.

Sampling design and statistical analysis

To minimize stress to the animals, we did not measure coloration and behavior for each individual within every stage. Instead, we aimed to obtain approximately 10 sets of color measurements and photographs and 20 behavioral trials per stage (Table 2). Within a reproductive cycle, we took color measurements of females no more than once per reproductive stage and used females in no more than 2 behavioral trials per stage. We took a total of 98 sets of reflectance measurements, analyzed 82 photographs, and conducted 186 behavioral trials (Table 2). This amounted to a mean \pm SD of 3.4 ± 1.8 spectral measurements, 2.8 ± 1.2 measures of color area, and 7 ± 3.8 behavioral trials per female per reproductive cycle for reproductive females and 1.1 ± 0.4 spectral measurements and 2 ± 0 behavioral trials per female for nonreproductive females.

We first tested whether there was a significant difference in body size (SVL) or condition of females that remained white and never reproduced throughout the breeding season, compared with females that turned orange and bred. Next, we tested whether female coloration or reproductive behavior differed significantly between reproductive stages using a generalized linear mixed model (GLMM; PROC GENMOD SAS version 9.1). To account for autocorrelated data due to repeated use of individuals and use of the same individual over more than one reproductive cycle, we included female ID and

female cycle (first, second, or third) nested within female ID as random factors in GLMMs. As males were also used multiple times in behavioral trials, we included male ID as an additional random factor when analyzing male behavior in relation to female coloration and behavior. We used least square differences (LSD) post hoc tests to test for differences between each pair of stages.

RESULTS

Only females that turned orange, and all those that did so, reproduced. There was a trend for females that developed orange coloration to be larger than those that stayed white ($F_{1,23} = 8.12$, $P = 0.09$); however, there was high overlap between the 2 groups. Females that stayed white had a mean (\pm SD) SVL of 53.4 mm (± 3.5 mm) and ranged from 49–59 mm SVL, whereas orange females had a mean SVL of 57.8 mm (± 3.5 mm) and ranged from 52–64 mm SVL. There was no significant difference between the body condition of females that did and did not turn orange ($F_{1,23} = 0.27$, $P = 0.61$).

Female color expression

The gular stripe turned from white to black between stages 1 and 2 and was darkest at stage 6 (Figure 1a,c). Achromatic (brightness) contrast between the gray or black stripe and surrounding white throat differed significantly among stages ($F_{8,55} = 11.65$, $P < 0.0001$). The gular stripe was significantly lighter (less achromatically contrasting) at stages 0 and 1 than every other stage (LSD post hoc tests: $P < 0.0001$ for all cases, Figure 1a) and darker at stage 6 than stages 0, 1, 2, 3, 4, and 8 (LSD post hoc tests: $P < 0.01$ for all cases). As white, gray, and black are chromatically very similar, chromatic contrast of the gular stripe against the white throat was low and relatively constant across the reproductive cycle (Figure 1c).

Orange coloration of the throat and abdomen had a clear secondary UV peak (Supplementary Figure S2c). The orange coloration of females varied significantly across the reproductive cycle (throat achromatic contrast: $F_{8,56} = 20.92$, $P < 0.0001$; throat chromatic contrast: $F_{8,56} = 25.99$, $P < 0.0001$; abdomen achromatic contrast: $F_{8,58} = 11.12$, $P < 0.0001$; abdomen chromatic contrast: $F_{8,58} = 18.07$, $P < 0.0001$; Figure 1b,c). Orange coloration began developing at stage 1 (by definition, see Table 1), rapidly increased through stage 1, reaching maximum intensity over stages 2, 3, 4, 5, 6 before fading rapidly over stages 7 and 8 (Figure 1b,c). Achromatic contrast of both the throat and abdomen was significantly higher in stages 2–7 than 0, 1, and 8 (LSD post hoc tests: $P < 0.01$ for all cases, Figure 1b), which did not differ significantly from each other. Chromatic contrast peaked over stages 2–5 for the throat and over stages 3–7 for the abdomen (Figure 1c) and was significantly higher at these stages than other stages (LSD post hoc tests: $P < 0.03$ for each of these comparisons; Figure 1c).

Consistent with the spectral qualities of female coloration, the proportion of orange coloration varied significantly among stages (throat: $F_{7,44} = 3.1$, $P = 0.009$; abdomen: $F_{7,44} = 6.16$, $P < 0.0001$; total area: $F_{7,44} = 10.13$, $P < 0.0001$; Figure 1d). Females had a significantly larger relative area of orange coloration in stages 3–6 than in stages 1, 2, 7, and 8 (LSD post hoc tests: $P < 0.05$ for each of these comparisons). Overall, female color expression reached its full intensity (in terms of both spectral properties and relative area) over stages 2–7 before fading just prior to or after laying (stages 6–8).

To test whether color expression might be an indicator of female body size, condition, or reproductive capacity, for each female that developed orange coloration and laid a clutch ($N = 17$ because 1 female died before oviposition), we examined the relationship between 3 variables and maximum color

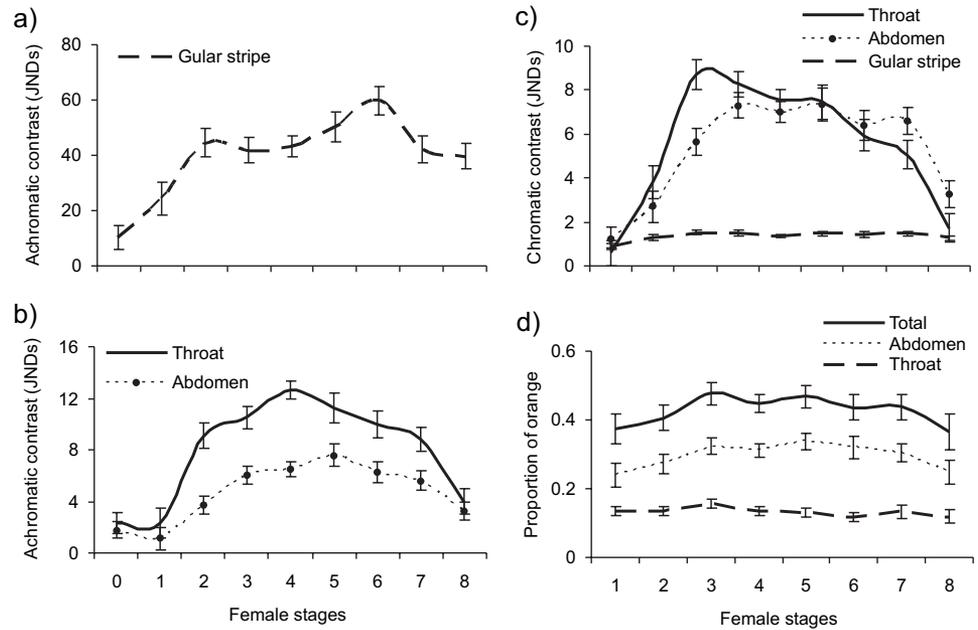


Figure 1

Mean (\pm standard error) of (a) achromatic (brightness) contrast of the gular stripe against the surrounding white throat; (b) achromatic contrast of the throat and abdomen; (c) chromatic (color) contrast of the throat, abdomen, and gular stripe; and (d) relative area of orange coloration on the throat, abdomen, and both combined (total) over the 8 stages defined in Table 1.

expression of the throat, which is most visible to conspecifics. Maximum color expression was defined as maximum relative area and achromatic and chromatic contrast (with the latter 2 derived from reflectance measurements on a single day between stages 3 and 6 for each female). Size and condition were measured prior to females entering their first ovarian cycle, and reproductive output was quantified as size and mass of the first clutch and size and mass of all clutches combined. We used multiple regression with stepwise selection ($P \leq 0.1$ as criterion for entering and staying in the model). Neither achromatic nor chromatic contrast of orange coloration was significantly correlated with female size, condition, or reproductive output (no variable met the $P < 0.1$ criterion for retention in the model). There was a trend for maximum relative area of orange throat coloration to be correlated with the total number of eggs laid by a female over the breeding season ($P = 0.054$, standardized coefficient = 0.28, $R^2 = 0.30$).

Female reproductive behavior

The frequency of female head bobs varied significantly throughout the reproductive cycle ($F_{8,112} = 2.10$, $P = 0.04$), and a similar marginally nonsignificant trend was evident for arm waves ($F_{8,112} = 1.93$, $P = 0.06$). Females performed significantly more head bobs and arm waves during stages 0, 7, and 8 than stages 2, 3, 4, and 6 (LSD post hoc tests: $P < 0.05$ for each of these comparisons). As both arm waves and head bobs appeared to vary with female coloration rather than among stages in which females were orange, we tested for an effect of color on the frequency of these 2 behaviors. We found that white-colored females performed significantly more of both behaviors than orange females (arm waves: $F_{1,119} = 10.57$, $P = 0.0015$; head bobs: $F_{1,119} = 9.63$, $P = 0.0024$; Figure 2).

Females primarily copulated soon after turning orange and significantly more often during stages 1, 2, and 3 than stages 0, 5, 6, and 7 (main effect of reproductive stage: $F_{8,112} = 4.18$, $P = 0.0002$; LSD post hoc tests: $P < 0.05$ for each of the previous comparisons; Figure 3a). No females copulated in stage 0 or 6, but 3 females copulated in stage 4, and 1 female copulated in stages 5 and 7, all of which appear to have been forced. Females also copulated in stage 8, where females were either

white again or possessed faded orange coloration prior to their next ovarian cycle.

Females performed 2 principal types of rejection behaviors, lateral displays, and flips, whose frequencies varied significantly across the reproductive cycle (lateral display: $F_{8,112} = 13.65$, $P < 0.0001$; flip: $F_{8,112} = 9.73$, $P < 0.0001$; Figure 3b,c, respectively). Females performed significantly more lateral displays during stages 3, 4, 5, and 6 than any other stages (LSD post hoc tests: $P < 0.01$ for each of these comparisons). Flips were performed significantly more frequently by females in stages 4, 5, and 6 than any other stages (LSD post hoc tests: $P < 0.05$ for each of these comparisons).

Male behavior

Males courted females by performing head bobs and harassed females by chasing and biting them. Male head bobs varied significantly across the reproductive cycle ($F_{9,118} = 4.06$, $P = 0.0001$) and increased dramatically between stages 1 and 2,

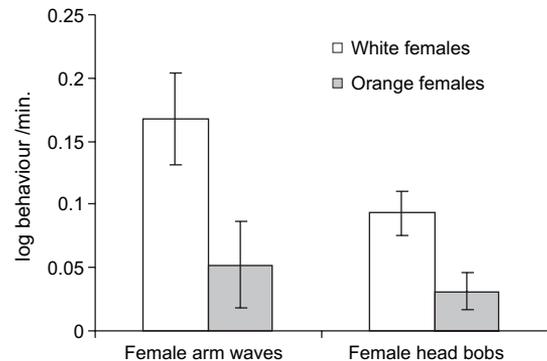


Figure 2

Mean (\pm standard error) of arm waves and head bobs performed by white- and orange-colored females. White females performed significantly more arm waves and head bobs than orange females ($P < 0.002$ in both cases).

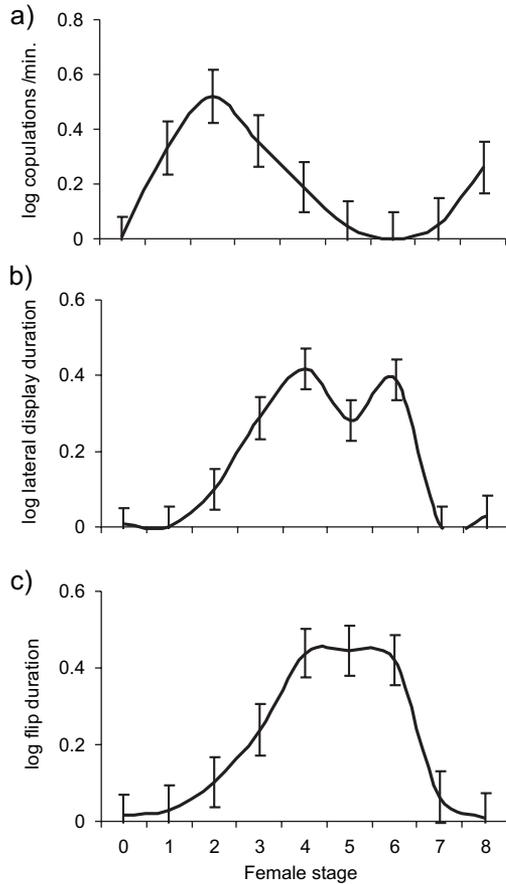


Figure 3 Mean (\pm standard error) frequency of (a) copulations, (b) lateral display, and (c) flip over performed over the 8 stages of the female reproductive cycle.

remaining at a similar level over subsequent stages. The frequency of head bobs during stages 2, 3, 4, 5, 6, 7, and 8 were significantly higher than stages 0 and 1 (LSD post hoc tests: $P < 0.03$ for all these comparisons). By contrast, variation in male bite duration across the reproductive cycle was marginally nonsignificant ($F_{8,112} = 1.87, P = 0.07$).

As both male head bobs and bite duration appeared to vary primarily with female coloration rather than among stages in which females exhibited orange coloration, we tested for an effect of color on the frequency of these male courtship and harassment behaviors. We found that males courted and harassed orange-colored females significantly more than white-colored females (head bobs: $F_{1,119} = 9.85, P = 0.0021$; bite duration: $F_{1,119} = 6.80, P = 0.01$; Figure 4).

We then tested whether male courtship and harassment behaviors varied in relation to female rejection behavior. Male head bobs were significantly positively related to the duration of both lateral displays and flips (lateral display: $F_{1,129} = 26.95, P < 0.0001$; flip: $F_{1,129} = 24.51, P < 0.0001$; Figure 5a,b), and male bite duration was significantly positively associated with female flip duration ($F_{1,129} = 5.16, P = 0.025$; Figure 5c). However, there was no significant relationship between male bite duration and female lateral display duration ($F_{1,129} = 0.08, P = 0.78$). Finally, in trials with successful copulations, females performed significantly fewer lateral displays and flips than in trials without copulations (lateral display: $F_{1,129} = 5.81, P = 0.017$; flip: $F_{1,129} = 8.74, P = 0.0037$; Figure 6).

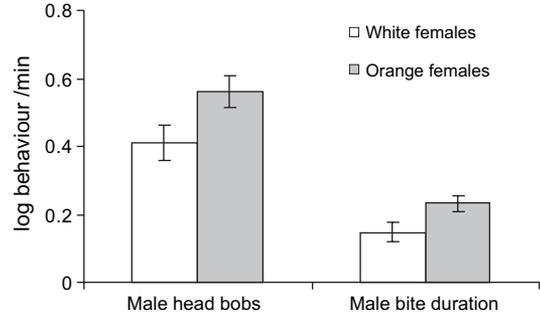


Figure 4 Mean (\pm standard error) male courtship and harassment behaviors (head bobs and bite duration, respectively) performed to white- and orange-colored females. Males courted and harassed orange females significantly more than white females ($P < 0.01$ in both cases).

DISCUSSION

We quantified female coloration across the female reproductive cycle and studied female and male behavior to examine the relationship between color expression and female reproductive status and to test whether female coloration increased

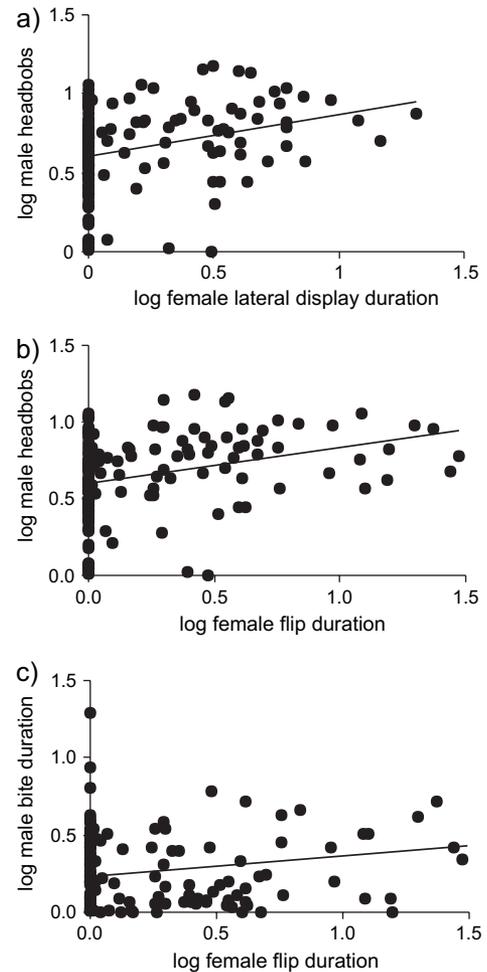


Figure 5 Positive relationships between duration of female rejection behaviors (lateral displays and flips) and male courtship (head bobs, $P < 0.0001$ in both cases) and positive relationship between female flip duration and male harassment (bite duration, $P = 0.025$).

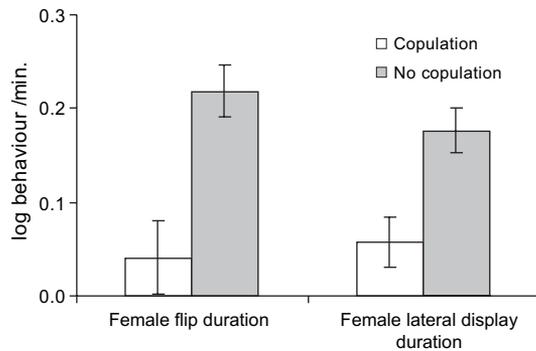


Figure 6
Mean (\pm standard error) female rejection behaviors (lateral displays and flips) performed in trials with and without successful copulations. Females performed significantly more rejection behaviors in trials that did not result in successful copulation ($P < 0.02$ in both cases).

or decreased male courtship. Females developed orange coloration as follicles began enlarging and color expression peaked around the inferred time of ovulation. Maximum color expression remained throughout the period when females were non-receptive (and behaviorally rejected males), until just prior to oviposition. Males courted orange females significantly more often than white females. However, males persistently courted and attempted copulation with unreceptive orange females despite clear behavioral rejection, suggesting that males may attempt forced copulation regardless of a female behaviorally signaling her receptive status.

Female color expression and reproductive status

Female *C. maculosus* exhibited bright orange ventrolateral coloration both when receptive and when unreceptive indicating that orange coloration does not function solely to stimulate courtship or signal rejection. In several species, coloration may signal both receptivity and nonreceptivity depending on intensity or pattern. For instance, in striped plateau lizards, female coloration becomes lighter after ovulation (Weiss 2002), whereas in the earless lizards, *Holbrookia maculata* (Hager 2001) and *Holbrookia propinqua* (Cooper et al. 1983); tree lizards, *Urosaurus ornatus* (Zucker and Boecklen 1990); and the tropidurid lizard *Microlophus occipitalis* (Watkins 1997) it intensified. Similarly, female common chameleons develop different color patterns to signal a change in receptive status (Cuadrado 2000). In these species, coloration appears to function as a conditional signal. In *C. maculosus*, however, orange coloration does not appear to correspond with receptive status because the relative area and intensity of orange coloration peaked between stages 2 and 3 when females appear to be receptive, judging by the higher frequency of copulations, and continued to be maintained when females were no longer receptive. Moreover, both female rejection behaviors (lateral aggressive displays and flipping) emphasize ventrolateral orange coloration. Several of the species mentioned above similarly show a gradation in female coloration with respect to reproductive status (Cooper et al. 1983; Zucker and Boecklen 1990; Watkins 1997; Hager 2001). These field-based studies did not quantify the timing of color change and ovulation in relation to copulation; therefore, the precise relationship between coloration and receptivity remains equivocal. In *C. maculosus*, it may be the combination of female coloration and rejection behaviors, rather than coloration per se, which allows males to distinguish whether or not

a female is receptive, thereby facilitating male decisions regarding courtship investment. However, this needs to be confirmed via experimental studies manipulating color independently of rejection behavior. Furthermore, male courtship and harassment did not decrease when females were gravid. This begs the question of why orange coloration is maintained when females are no longer receptive because it is associated with prolonged courtship and harassment.

There are at least 2 potential reasons for maintenance or intensification of coloration after receptivity. First, females may be physiologically unable to uncouple color expression and reproductive condition (Cooper 1984). In *C. maculosus* as in other lizards, color expression is associated with plasma steroid concentrations (Jessop et al. 2009). Rather than decreasing after ovulation, plasma concentrations of progesterone and testosterone remain high until oviposition (Jessop et al. 2009). Because females maintain high steroid concentrations throughout the reproductive cycle and steroid concentrations are associated with color expression (Jessop et al. 2009), females may be physiologically unable to reduce color expression when unreceptive. Second, males may be familiar with the dynamic color expression of females on his territory such that orange coloration, in combination with rejection behaviors, may reduce courtship and harassment from familiar males. Additionally, maintenance of conspicuous orange coloration may be less costly than previously assumed (see Olsson 1995a) if the abundance of predators in barren salt pan habitats is low or predators avoid orange females.

Costs and benefits of female rejection and persistent male courtship

We found a positive relationship between the frequencies of male head bobs and both female lateral displays and flips and between male bite duration and female flip duration. This suggests that the more persistently males court and harass females, the more females resist male courtship and copulation attempts. However, in trials where females performed more rejection behaviors, there were fewer successful copulations suggesting that despite persistent courtship and copulation attempts by males, female rejection behaviors successfully reduced the number of copulations. This may minimize risk of injury, thereby reducing potential direct costs of mating. During copulation, the male bites the female's nape, which can result in injury. Death due to copulation has been observed in *C. maculosus*, where the female's spine was penetrated by the male's teeth (Olsson 1995a). The risk of injury is therefore greatest during copulation and attempted forced copulation. This suggests that direct costs of copulation in *C. maculosus* are potentially high and may explain why females have evolved an apparently very costly rejection behavior (Olsson 1995a).

Persistent courtship is likely to be energetically costly for males; however, males may gain fitness benefits from persistent courtship or forced copulations with unreceptive females if females store sperm between ovarian cycles, as is the case in *C. maculosus* (Mitchell 1973; Olsson 1995a). Although forced copulations are likely to be costly to females, in some species, females are able to retaliate via postcopulatory cryptic female choice to minimize the fitness benefits to males (Pizzari and Birkhead 2000). Furthermore, even though aggressive rejection may be just as energetically costly as copulation, females may potentially gain indirect genetic benefits from rejecting all but the most persistent males (Chapman et al. 2003; Kokko et al. 2003). Persistent male courtship may also indicate male quality (e.g., Stapley 2008) and therefore be used by females as a cue for mate choice during subsequent ovarian cycles.

Our results suggest that females use both color and behavioral signals to reduce courtship when females are not in

reproductive condition. Males courted unreceptive white females significantly less often than orange females, and arm waves and head bobs were primarily performed by nonreproductive females prior to turning orange and after oviposition when orange coloration faded. Male agamids have been shown to complete testis development prior to or very soon after emergence from winter inactivity (Niejalke 2006). As male *C. maculosus* emerge earlier than females (Mitchell 1973), they are already reproductively primed when females first emerge; therefore, signaling nonreproductive condition is likely to reduce male harassment during periods when females are not physiologically and behaviorally ready for mating.

A role for sexual selection?

In addition to signaling aspects of reproductive status, female-specific ornamentation may function in male mate choice or female–female competition. However, aggression between females has not been observed in the wild during detailed, long-term ecological studies of the species (Mitchell 1973; Olsson 1995a, 1995b). Moreover, we found little support for a relationship between maximum color expression of the throat (in terms of either spectral qualities or relative area) and female size, condition, or reproductive output. We only found a weak correlation between the maximum relative area of orange coloration (relative to body size) during the first ovarian cycle and the total number of eggs laid by a female over the breeding season. Nevertheless, as the absolute area of orange coloration (rather than the relative area) is greater in larger females, the absolute area of orange coloration could potentially facilitate assessment of female size and condition, that is, orange coloration could act as an amplifier (Maynard Smith and Harper 2003). There is some evidence for male mate choice based on female coloration in other lizards (e.g., LeBas and Marshall 2000; Weiss 2006). The role of orange coloration in male mate choice warrants further investigation.

CONCLUSIONS

Our results suggest that in Lake Eyre dragons, female ornamentation signals reproductive status as only orange females reproduced and males courted orange females more than white females. However, the orange coloration is maintained at similar intensity when females are both receptive and unreceptive and emphasized during rejection displays. These results are neither inconsistent with either the courtship stimulation or courtship rejection hypotheses nor support the view that color functions as a conditional signal. Instead, 2 possible explanations for the maintenance of orange coloration throughout the reproductive cycle, despite persistent male courtship and harassment are that 1) female color expression and reproductive condition or behavior share the same underlying physiological basis and consequently may be evolutionarily difficult to uncouple and 2) females gain indirect genetic benefits from stimulating male courtship as well as from apparently costly resistance by mating only with the most persistent males (Chapman et al. 2003; Kokko et al. 2003). Additionally, the predation cost of exposing conspicuous orange coloration during rejection displays may be much lower than previously assumed in the barren habitat occupied by this species (McLean C, Stuart-Fox D, unpublished data).

Female ornamentation is widespread across diverse taxonomic groups, including many species with conventional sex-roles (Amundsen and Forsgren 2001). Despite this, for the great majority of species, relatively little is known regarding its function and evolution. In species with conventional sex-roles, female ornamentation may have evolved to signal

multiple facets of a female's reproductive status and quality. In *C. maculosus*, the area and intensity of orange coloration varied considerably among females and could potentially facilitate male mate choice and decisions regarding courtship investment, in addition to assessment of female reproductive status. Our study highlights the need for additional research on the fitness benefits, as well as the costs and physiological constraints associated with female color expression, in order to understand the ecological and social conditions favoring the evolution of female ornamentation.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>.

FUNDING

Australian Research Council (DP0772215); University of Melbourne (ECR 600065).

We thank Adnan Moussalli for assistance in the field, Mats Olsson for advice and expertise through the course of the project, and Cindy and Trevor Mitchell for their hospitality at Muloorina Station. South Australian Department of Environment and Heritage permit to undertake scientific research S25421_1; Wildlife Ethics Committee approval 13/2007; University of Melbourne Animal Ethics Committee approval 0701400.1.

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