

Signalling with physiological colours: high contrast for courtship but speed for competition



Anuradha Batabyal, Maria Thaker*

Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

ARTICLE INFO

Article history:

Received 30 January 2017
Initial acceptance 1 March 2017
Final acceptance 21 April 2017
Available online 24 June 2017
MS. number: 17-00106R

Keywords:

agama
aggression
colour change
courtship
lizard
social interaction
urbanization
visual signal

Dynamic physiological colour change allows animals to alter colours and patterns for communication, camouflage and thermoregulation. Using reflectance spectrometry and digital photography, we found that males of the Indian rock agama, *Psammophilus dorsalis*, can rapidly express intense colours that are different from the neutral state and specific to the social context. The distinct bands on males shifted between yellow and red (dorsal) and between orange and black (lateral) within seconds, and the resulting colour pattern was diametrically different depending on whether males were in courtship or aggressive interactions. Although males showed higher chromatic contrast when courting females, the colour change was faster during competitive encounters with other males. The nature of this social colour communication also differed across populations in anthropogenically disturbed landscapes. Compared to males from rural areas, suburban males were slower to change colour and showed duller and paler colours during staged social encounters. Consistent with other disturbance-induced shifts in phenotypic traits seen in numerous taxa, we provide the first evidence that social signalling through dynamic colour change in terrestrial vertebrates is also affected by urbanization.

© 2017 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Dynamic physiological colours, which are actively modulated by signalers within seconds to minutes, have been documented in a wide range of taxa from crustaceans and cephalopods to fishes, amphibians and reptiles (Camargo, Visconti, & Castrucci, 1999; Hanlon, 2007; Hemmi, Marshall, Pix, Vorobyev, & Zeil, 2006; Stuart-Fox & Moussalli, 2008; Zylinski, How, Osorio, Hanlon, & Marshall, 2011). Capacity of physiological colour change, however, varies dramatically between species. Aquatic taxa, such as cephalopods and fishes, show remarkable abilities to change body colour and pattern within seconds to minutes (Messenger, 2001; Morris, Mussel, & Ryan, 1995). For most terrestrial species, these physiological colour changes are limited to achromatic changes in contrast with lightening or darkening of the skin (e.g. Camargo et al., 1999; Cooper & Greenberg, 1992; Kindermann, Narayan, Wild, Wild, & Hero, 2013). For a few well-characterized terrestrial vertebrates, physiological colours can shift hues across a wider range of visible wavelengths. In the Chamaeleonidae family, many species are capable of large changes in both achromatic contrast and chromatic contrasts in the red, brown, yellow and green spectral wavelengths

(Ligon & McGraw, 2013; Stuart-Fox & Moussalli, 2008; Teyssier, Saenko, Van Der Marel, & Milinkovitch, 2015). Regardless of the specific colour patterns, physiological colour changes for both aquatic and terrestrial taxa depend on context, shifting for camouflage, thermoregulation and social communication (Messenger, 2001; Stuart-Fox & Moussalli, 2009; Zylinski et al., 2011).

Understanding variation in colour signals has been central to the study of animal communication for decades (Andersson & Andersson, 1998; Dale, 2006; Hamilton & Zuk, 1982). Given that signals have evolved to attract attention and convey information (Hebets & Papaj, 2005; Partan & Marler, 2005), species that use physiological colours for communication are able to use certain colour patterns to convey specific information in different social contexts. For example, in three-spined sticklebacks, *Gasterosteus aculeatus*, males shift the intensity of their red nuptial coloration during both male–male and male–female interactions to convey dominance status and male parental ability (Candolin, 2000). Dwarf chameleons, *Bradypodion* sp., are observed to alter their chromatic contrast to signal dominance or submissiveness during social interactions, regardless of their conspicuousness against the background (Keren-Rotem, Levy, Wolf, Bouskila, & Geffen, 2016; Stuart-Fox & Moussalli, 2008). Dynamic signals can also be used to convey different information to different receivers. Male

* Correspondence: M. Thaker, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India.

E-mail address: mthaker@ces.iisc.ernet.in (M. Thaker).

mourning cuttlefish, *Sepia plangon*, can lateralize physiological colour changes, such that one side expresses male courtship patterns, while the other expresses female-mimicked patterns. This dual signalling state allows males to court females, while simultaneously distracting a rival male (Brown, Garwood, & Williamson, 2012). Given that a single body region can express more than one chromatic and achromatic state at any given point in time, signalers that use physiological colours have the potential to convey multiple messages rapidly and dynamically during courtship and aggressive encounters.

Most studies on physiological colours have illustrated the importance of environmental conditions, focusing on induced patterns to enhance crypticity or conspicuousness against different substrates or backgrounds (Clarke & Schluter, 2011; Cole & Endler, 2015; Endler, 1992; Stuart-Fox & Moussalli, 2009; Wong & Candolin, 2015). Individuals should also vary the intensity and brightness of colour patterns for social communication depending on local environmental conditions (e.g. Kelley, Phillips, Cummins, & Shand, 2012; Leal & Fleishman, 2003). For example, in three-spined sticklebacks, algal turbidity influences the expression of the red nuptial coloration, depending on whether a male is poor or good quality (Wong, Candolin, & Lindström, 2007). Similarly, differences in the intensity of light in xeric and mesic habitats seem to influence the brightness of the statically coloured dewlaps on *Anolis cristatellus* (Leal & Fleishman, 2003). Surprisingly very few terrestrial vertebrates have been studied to determine intraspecific population variation in physiological colours across different environmental conditions (but see Boback & Siefferman, 2010). Because they are dynamic, and thus a form of phenotypic plasticity, use of physiological colours can respond to external drivers over very short timescales, and provide a good opportunity to examine how changes in both the physical and social environment influence intraspecific variation in magnitude and range of colour patterns.

Here, we examined the pattern, range and speed of physiological colour change in the Indian rock agama, *Psammophilus dorsalis*, to determine how visual signalling differs between social context and between populations. We explicitly quantified colour changes during social interactions with females as well as males to determine whether this species uses different visual signals for courtship and aggression. *Psammophilus dorsalis* is widely distributed in semiarid open habitats of southern peninsular India, which include human-modified environments. To quantify the range (i.e. capacity) of physiological colour changes during social interactions for this species, we included males from both suburban and rural areas, representing two contrasting environmental conditions. Urbanization alters a large suite of biotic and abiotic factors, from lighting conditions and habitat composition to levels of social competition and predation risk (Kempnaers, Borgström, Loës, Schlicht, & Valcu, 2010; Longcore & Rich, 2004). Mounting evidence suggests that these changes can affect animal communication by affecting the production of signals, transmission efficiency and receiver reception (Rosenthal & Stuart-Fox, 2012). In this paper, we focus on the physiological colour signals themselves, to determine strategies and variation in their use. In doing so, we examine the relative importance of social context and environment in the expression of physiological colour patterns.

METHODS

Study Species and Areas

Psammophilus dorsalis occurs in semiarid habitats, where they are found on rocky boulders and sheet rocks interspersed with scrub vegetation (Radder & Saidapur, 2005), as well as in suburban

habitats (Balakrishna, Batabyal, & Thaker, 2016). In the latter, *P. dorsalis* are generally found in localized clusters around residential construction sites, unbuilt plots and urban gardens. *Psammophilus dorsalis* is sexually dimorphic and males are larger than females (snout–vent length: males = 95–140 mm and females = 75–96 mm). Males also develop conspicuous physiological colours during the breeding season, typically from April to August (Radder & Saidapur, 2005), which become dull at the end of the breeding season.

Adult lizards were collected from study sites of approximately 1 km² each, located in and around the city of Bangalore, India. Suburban study sites ($N = 3$ sites) were located within city limits and rural study sites were located near Antharagange forest range in Kolar district ($N = 2$ sites). Replicate sites within each habitat were 5–10 km apart, and the suburban and rural habitats were at least 60 km apart. We performed the following experiments during the peak breeding season (April–August) in 2013 and 2014. This species is known to court and fight throughout the breeding season (Deodhar, 2017; Radder, Saidapur, Shine, & Shanbhag, 2006) and has one or two clutches a year (Srinivas, Hegde, Sarkar, & Shivanandappa, 1995), and thus were expected to be socially responsive during the experimental trials. Adult male lizards [$N = 25$ suburban (7–10 per site) and 25 rural (10–15 per site)] were captured by noosing and brought into the laboratory. All males and females used in the study were sexually mature adults. We ensured that all females were nongravid to minimize variation in receptivity: all females were behaviourally reactive during social trials (e.g. responded to males with head bobs and tail raise).

Wild-caught lizards were housed individually in glass terraria in a dedicated animal housing room that permitted natural temperature and light conditions. Terraria (60 × 30 cm and 25 cm high) were lined with disposable paper towels as the substratum, provided with rocks for refuge, and were covered on all sides to minimize disturbance. Individual incandescent basking lights (60 W), above each terrarium, were turned on from 0800 to 1200 hours, and from 1500 to 1600 hours. Lizards received live ants and field crickets daily for food and water ad libitum and were maintained in the laboratory for 7–9 days. Before the start of the experimental trials, mass (g) and snout-to-vent length (mm) were measured for all individuals using a weighing balance and digital callipers, respectively. All lizards were returned to the site of capture after the experimental trials.

To quantify the effect of social stimulus on colour patterns, we exposed each focal male ($N = 20$ total suburban and 20 rural, excluding control animals) to a stimulus male and female separately. Stimulus individuals were caught from rural and suburban sites that were different from where focal animals were caught, and therefore were unfamiliar to focal males. Each stimulus individual was randomly paired with two or three focal individuals from the same population. Social interactions were staged under full-spectrum lights (Viva-Lite:B22) in test tanks (95 × 45 cm and 30 cm high), and began with a 30 min acclimatization period, followed by a 30 min interaction period with one of the stimuli. Males in the control treatment ($N = 5$ suburban and 5 rural) were also kept in the same test tank for 30 min but were not exposed to any social stimuli. Lizards were randomly assigned to control or social test groups, where the order of stimulus (exposure to female or male) was also randomized. All trials were conducted from 0800 to 1200 hours or from 1500 to 1700 hours, which are the peak activity periods for this species in our study areas. Test tanks were kept behind a blind which enabled us to digitally record the social interactions with minimal disturbance (using a Canon EOS550D with 18–55 mm lens) for subsequent quantification of the speed of colour changes (see details below).

Body Colour Quantification

We quantified physiological body colour changes in two ways, using reflectance spectrometry and standardized photography. During the 30 min social interactions and for control males (no social interactions), we took repeated measurements of percentage reflectance of the dorsal and lateral sides of the body. Reflectance measurements of a 2 mm² area of skin surface were taken through a custom-made flexible fibre-optic probe, angled at 45° relative to the body, that was connected to a spectrometer (JAZA2474, Ocean Optics, Dunedin, FL, U.S.A.) and light source (PX lamp, Ocean Optics). The PX lamp triggering rate was set at 10 ms, boxcar at 5 and each measurement was averaged over three spectra readings. Spectral measurements for each individual were first corrected against white and black standards (as per White et al., 2015). Because colour changes occur rapidly in this species and are affected by stress and handling, we mounted the probe against the body of the lizard with Velcro tape before the start of the acclimation period (see Fig. 1). This enabled us to take repeated measurements of colour without handling the lizard and disturbing the social interactions.

Thus, for each focal male, we recorded spectral reflectance of the dorsal and lateral body regions at least six times at 5 min intervals during the 30 min social interaction period. Reflectance spectra from each time point were averaged across every 5 nm wavelength range and we selected the maximum colour spectra from each individual for subsequent visual modelling. We chose the maximum spectra because we wanted the highest magnitude of colour change expressed. Chromatic (dS) and achromatic (dL) contrasts from these spectra were calculated to determine whether the maximum spectra could be discriminated in the perceptual colour space of an agamid lizard, as defined by the quantum catches of receptors in cone cells, taking spectral sensitivity of each cone receptor type and receptor noise of a typical agamid eye into account (as per Barbour et al., 2002; Rankin & Stuart-Fox, 2015; Teasdale, Stevens, & Stuart-Fox, 2013; Yewers et al., 2015). Model calculations are detailed in Teasdale et al. (2013). Background reflectance for all data was standardized against a brown background and irradiance conditions were set as standard sunlight without shade. Thus, for each individual, we calculated the maximum change in chromatic and achromatic contrast values for each body part (dorsal and lateral) during male–male interactions, male–female interactions and control conditions.

To quantify the speed of the maximum physiological colour change during social interactions, we used the digital videography data and extracted images of the focal male at 10 s intervals. For each image, we calculated hue and chroma values (see details below) and used these colour parameters to calculate the speed of the maximal shifts in colour for dorsal and lateral bands in both social contexts for suburban and rural males. Methods for hue and chroma calculations from photographs were as per Stevens, Parraga, Cuthill, Partridge, & Troscianko, 2007 (see also Smith et al., 2016). In brief, a photograph of a colour checker (X-rite model: MSCPP), with grey/white/black card with 20% grey reflectance was taken with the same camera under the same lighting conditions to linearize and equalize photographs before analysis (Stevens et al., 2007). From all digital images, we extracted the red (R), green (G) and blue (B) values of a standard-sized square patch (0.5 cm) of the dorsal and lateral body regions using Matlab script written by J. Endler (Mathworks, Natick, MA, U.S.A.). The R, G and B values were used to calculate hue and chroma (as per Stevens et al., 2007; Smith et al., 2016). The time interval of the maximum change in hue (shift between yellow and orange-red on dorsal band) or chroma (shift between orange and black on the lateral band) was calculated as the speed of physiological colour change.

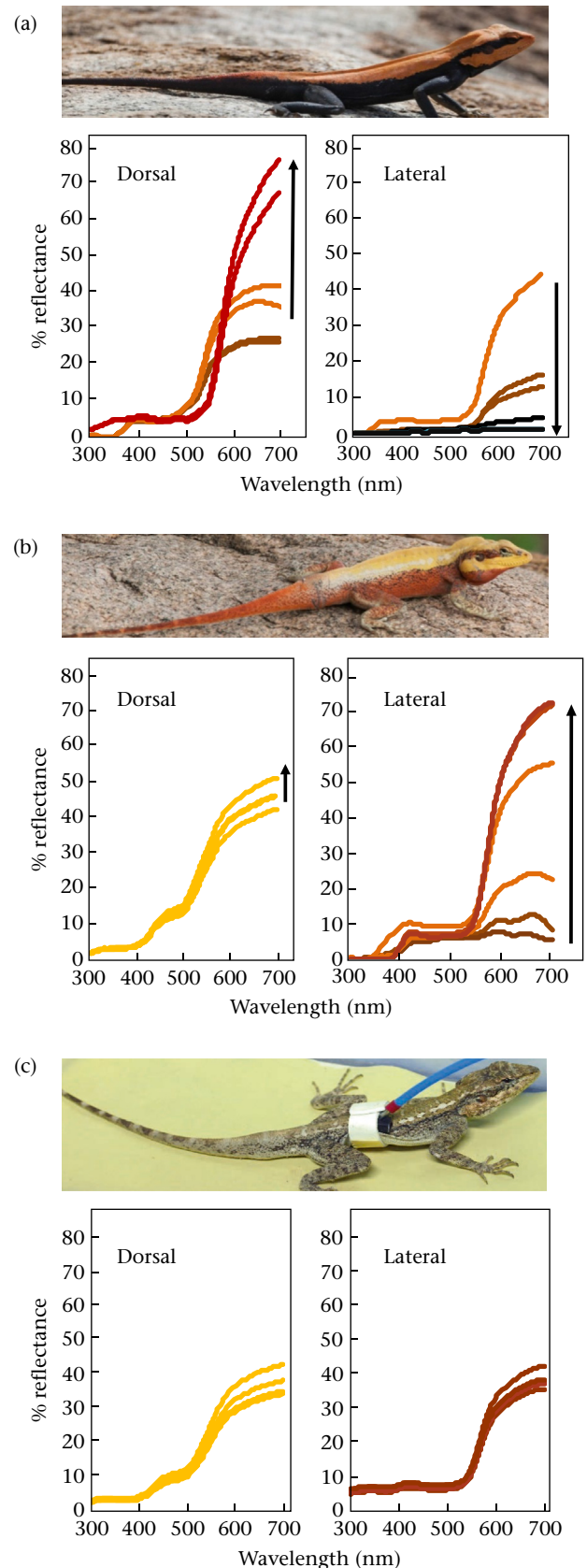


Figure 1. Physiological colour change in a typical male of *P. dorsalis* during social interactions. (a) Male–female interactions. (b) Male–male interactions. (c) Control nonsocial conditions. The arrows indicate direction of colour change over a 30 min interval.

Ethical Note

This species is not covered under the Schedules of the Indian Wildlife (Protection) Act; therefore, collection permits are not required. All capture, handling and experimental protocols were approved by the Institutional Animal Ethics Committee at the Indian Institute of Science (CAF/Ethics/394/2014).

Statistical Analyses

We compared the chromatic and achromatic contrasts of dorsal and lateral bands separately, to determine whether social context (male–male, male–female, control), population (suburban and rural) and body condition (see below) affected the magnitude of colour expression, using a linear mixed-effects model, with replicate sites and individual focal male ID as random effects (R package: lme4 and lmerTest: Bates, Maechler, Bolker, & Walker, 2014; Kuznetsova, Brockhoff, & Christensen, 2015). Body condition was calculated from mass and snout–vent length as the scaled mass index based on the central principle of scaling (see Peig & Green, 2009). We also compared the rate of maximal colour change on the dorsal and lateral bands to determine differences across social context (male–male, male–female), population (suburban and rural) and body condition using a linear mixed-effects model, with replicate sites and individual focal male ID as random effects. For all these analyses, we included an interaction term between social context and population and used the *F* test for lmer results (type III with Satterthwaite approximation for degrees of freedom). Where relevant, we performed post hoc comparisons for all interaction effect terms using Tukey contrasts (R package multcomp: Hothorn, Bretz, & Westfal, 2013). Variation in colour responses between replicate sites within populations (SD was < 0.5 for all tests) and between individual focal males (SD was < 2 for all tests) were negligible, and thus we pooled population data for all figures. All data were analysed in R version 3.0.3 (R Core Team., 2016).

RESULTS

During staged interactions with females, 19 rural males and 15 suburban males (of 20 each) showed colour changes on their dorsal and lateral bands that were at least four times greater than that seen during control conditions (chromatic contrast range for controls = 0.10–0.70 dorsal; 0.42–0.60 lateral). During staged interactions with males, 14 of 20 rural males and 11 of 20 suburban males showed colour changes that were also at least four times greater than during control conditions. Subsequent analyses of dorsal and lateral colour changes were restricted to only those lizards described above, as they responded to social interactions with detectable colour changes.

We found no significant effect of body condition on the chromatic and achromatic contrast of either the dorsal (chromatic: $F_{1,7} = 0.002$, $P = 0.961$; achromatic: $F_{1,26} = 0.317$, $P = 0.577$) or lateral (chromatic: $F_{1,41} = 0.004$, $P = 0.948$; achromatic: $F_{1,41} = 2.255$, $P = 0.065$) body regions for both populations in all social contexts. Body condition also did not affect the rate of either dorsal or lateral colour change (dorsal: $F_{1,32} = 0.331$, $P = 0.568$; lateral: $F_{1,27} = 0.078$, $P = 0.786$). Instead, we found that the physiological colours on both the dorsal and lateral bands of males changed independently depending on social context, with some variation between populations (detailed results below).

Magnitude of Physiological Colour Change

In general, when males of *P. dorsalis* interacted with females, the dorsal band changed from patchy yellow (typical neutral colour) to

orange or red, while the lateral band changed from patchy orange (typical neutral colour) to black (Fig. 1a). During interactions with other males, the dorsal band changed to yellow, while the lateral band changed to an even brighter orange (Fig. 1b). Under control conditions with no social encounters, the dorsal band remained a patchy or dull yellow-brown while the lateral band remained dull orange-brown, with little change during the 30 min observation period (Fig. 1c).

Regardless of the specific hues that were expressed, chromatic contrasts of the colours on the dorsal band and the lateral band were affected by an interaction between social context and population (Fig. 2; dorsal: $F_{2,23} = 3.18$, $P = 0.060$; lateral: $F_{2,41} = 4.87$, $P = 0.012$). Chromatic contrast of the dorsal band in rural males was highest during male–female interactions compared to male–male interactions (post hoc $z = -3.58$, $P = 0.004$) or control conditions (post hoc $z = 6.79$, $P < 0.001$). Chromatic contrast of rural males during male–male interactions was also significantly higher than during control conditions (post hoc $z = 2.91$, $P = 0.040$). Among suburban males, chromatic contrast of the dorsal band was not significantly different between social contexts (post hoc $z = -0.62$, $P = 0.988$), but was higher than that seen during control conditions (male–male: post hoc $z = 2.81$, $P = 0.054$; male–female: $z = 3.76$, $P = 0.002$). Rural and suburban males differed significantly in the chromatic contrast of their dorsal band during male–female interactions only ($z = -3.42$, $P = 0.007$). Thus, during interactions with females, rural males turned a richer shade of red (hue: 675 ± 4.82 SE; chroma: 2.43 ± 0.16 SE) compared to suburban males (hue: 667 ± 2.77 SE; chroma: 2.00 ± 0.05 SE). For the lateral band, rural males increased their chromatic contrast significantly during male–female interactions compared to during male–male interactions ($z = -5.71$, $P < 0.001$) and control conditions ($z = 6.59$, $P < 0.001$). Urban males increased the chromatic contrast of their lateral band similarly during both social interactions compared to control conditions (male–female: $z = 4.69$, $P < 0.001$; male–male: $z = 3.79$, $P = 0.002$). No significant difference was observed between populations in the chromatic contrast of the lateral band.

Achromatic contrast (brightness) of the dorsal band was significantly affected by social context ($F_{2,33} = 9.84$, $P < 0.001$): during male–female interactions it was significantly higher than in control conditions ($z = 3.46$, $P = 0.001$). No significant differences in achromatic contrast of the lateral band were seen across social contexts or between populations.

Speed of Physiological Colour Change

Speed of the maximal colour change on the dorsal band was affected by an interaction between social context and population ($F_{1,3} = 26.137$, $P = 0.011$). Both rural and suburban males changed the colour of their dorsal band significantly faster during male–male than male–female interactions (rural: $z = -2.90$, $P = 0.017$; suburban: $z = -13.25$, $P < 0.001$; Fig. 3a), but population differences in the speed of colour change was only apparent during male–female interactions: rural males were significantly quicker to change their dorsal band than suburban males (dorsal: $z = 5.07$, $P < 0.001$). Although all males initiated colour change within 10 s during any social interaction, the time taken by suburban males to maximally change their dorsal colour during interactions with females ranged from 260 to 1200 s whereas the longest time taken by a rural male to maximally shift its dorsal colour was 400 s (Fig. 3a).

Speed of the maximal colour change on the lateral band was affected independently by population and social context (population: $F_{1,18} = 7.287$, $P = 0.014$; social context: $F_{1,9} = 23.628$, $P < 0.001$). Similar to dorsal colour change, speed of the colour change on the lateral band was also significantly faster during

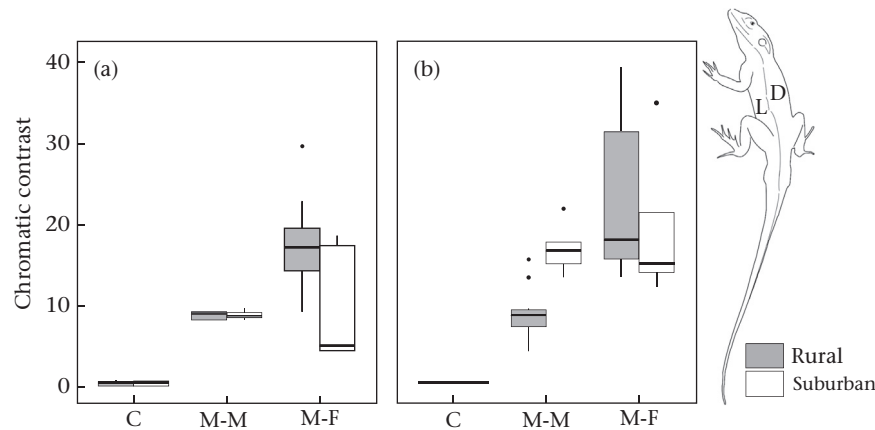


Figure 2. Chromatic contrast of the maximal physiological colour on the (a) dorsal band (D) and (b) lateral band (L) during control conditions (C), male–male interactions (M-M) and male–female interactions (M-F) for rural males (grey boxes) and suburban males (white boxes) of *P. dorsalis*. Box plots show medians, quartiles, 5th and 95th percentiles and extreme values.

male–male than male–female interactions and rural males changed colour faster than suburban males (Fig. 3b).

DISCUSSION

We found conspicuous and rapid colour change in an agamid lizard species, which involves independent shifts in colour on two body regions during social interactions. Not only did we add a new genus to the list of animals that utilize physiological colours, we showed that this colour change is dramatic, among the most rapid and specific to the social context. During encounters with females, the dorsal band on males of *P. dorsalis* physiologically changed from dull yellow to bright orange-red and the lateral band shifted from patchy brown to black. During encounters with other males, the dorsal band became bright yellow and the lateral band shifted to orange. These colour patterns resulted in diametrically different levels of chromatic contrast, such that males showed higher chromatic contrast overall when courting females than when interacting with other males. The colour changes were faster, however, during male–male than male–female interactions. Although it is common to find population differences in static colours (Endler, 1991; Marshall, Philpot, Damas-Moreira, & Stevens, 2015), we also found that the nature of this social colour communication differed between populations in anthropogenically disturbed landscapes, suggesting that variation in physiological colours is influenced by environmental conditions as well as social context.

Many animals use visual signals during social communication, typically showing context-specific displays for courtship and competition. In many agamid lizards, static or developmentally fixed colours affect the probability of winning intraspecific competitive encounters (Hamilton, Whiting, & Pryke, 2013; Healey, Uller, & Olsson, 2007; Stuart-Fox & Johnston, 2005) or attracting mates (e.g. Hamilton & Sullivan, 2005; LeBas & Marshall, 2000). Unlike static colour signals, however, which cannot change during social interactions, physiological colours provide a wider communication range for species. During social interactions, males of the panther chameleon, *Furcifer pardalis*, shift their skin colour patches to an excited state during both courtship and competition contexts (Teyssier et al., 2015). We found that males of *P. dorsalis* show rapid colour changes similar to those of chameleons, but have two distinct bands on their body, as opposed to irregular patches, that change during social interactions. Despite the numerous descriptions of colour changes in agamas (e.g. Anibaldi, Luiselli, & Angelici, 1998; Inoué & Inoué, 1977; Langkilde & Boronow, 2012;

Norfolk, Melotte, Gilbert, Zalat, & Reader, 2010; Smith et al., 2016), no study has found different colour patterns during courtship and aggressive interactions. Males of *P. dorsalis* used the same body regions to signal differently to males and females, shifting within seconds to minutes across large values of percentage reflectance (from 20 to 80%) along a wide visible spectral range (hue). Thus, physiological colour change is a major signalling modality in this species. The strikingly different colour patterns expressed suggest that males may be conveying different information to conspecific males and females.

Visual signalling strategies of *P. dorsalis* involve both contrast and speed. Colour changes on males of *P. dorsalis* showed highest chromatic contrast during courtship interactions, but were faster (with lower chromatic contrast) during aggressive interactions. Bright and intense colours with high contrast are considered preferred qualities of potential mates for many vertebrates (e.g. Dubuc, Allen, Maestripieri, & Higham, 2014; Fukuda & Karino, 2014; Hill, 2006), but the most extensive evidence comes from studies of species with static colour patches. Our results suggest that high chromatic contrast in colours during courtship displays (red dorsal + black lateral) may also be a signal of male quality for females. Conversely, the chromatic contrasts of colour patterns during aggressive encounters (yellow dorsal + orange lateral) and during neutral nonsocial contexts (patchy yellow dorsal + patchy orange-brown lateral) are both low, which may enable males to more rapidly shift from neutral to aggressive displays. Rate of colour change may be a signal of male dominance in *P. dorsalis*, similar to *Chameleo calyptratus*, in which the rate of colour change is correlated with winning fights (Ligon & McGraw, 2013). Determining the specific information conveyed by the speed and the colour patterns used during social interactions needs further investigation.

We found not only that *P. dorsalis* expresses rapid colour change that is specific to the social context, but also some population variation in the timing and magnitude of colour change that may be related to urbanization. Phenotypes of species that live in urban environments show major shifts in a range of ways, including changes in physiological, behavioural and morphological traits (French, Fokidis, & Moore, 2008; Hutton & McGraw, 2016; Lazić, Kaliontzopoulou, Carretero, & Crnobrnja-Isailović, 2013; Sol, Lapiedra, & González-Lagos, 2013). Effects of urbanization on animal communication has also been documented, especially in song structure and plumage colour of avian species and in social signalling in fish species (Jones, Rodewald, & Shustack, 2010; Nemeth

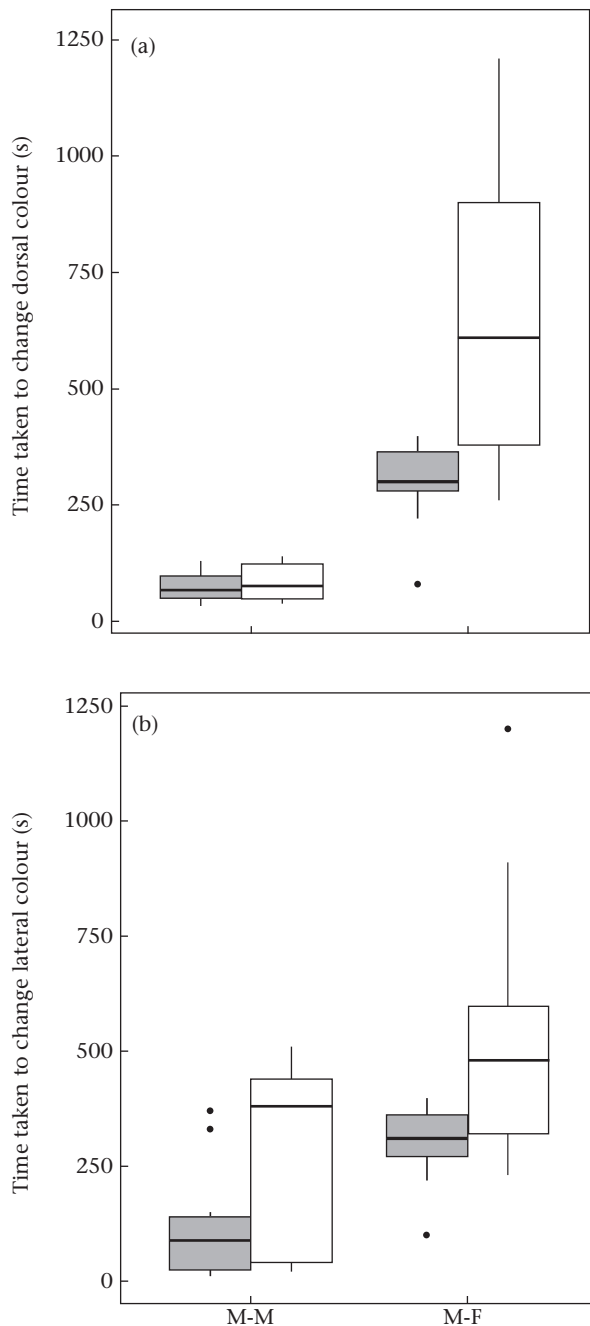


Figure 3. Time taken (s) to attain maximal colour shift on the (a) dorsal band and (b) lateral band during male–male interactions (M–M) and male–female interactions (M–F) for rural males (grey boxes) and suburban males (white boxes) of *P. dorsalis*. Box plots show medians, quartiles, 5th and 95th percentiles and extreme values.

et al., 2013; Tringali & Bowman, 2015; Wong & Candolin, 2015; Wood & Yezerinac, 2006). The effect of urbanization on visual signalling in lizards is unknown (but see Bateman & Fleming, 2014; Hutton & McGraw, 2016; Sol et al., 2013; Tuomainen & Candolin, 2011 for urbanization effects on other behaviours). We found that during courtship encounters, male lizards in suburban habitats showed duller colours and were generally slower to attain maximal colour shifts than rural males. If courtship colour changes are honest indicators of male quality, then the duller suburban males may be of lower quality than rural males, beyond the expected differences due to body condition. Furthermore, population differences were not similar for all colour bands on males, suggesting

that some signals (black lateral band) are more robust to environmental variation than others.

The intensity of visual colour signalling can be influenced by mechanistic as well as ecological and social factors. Yellow-red pigment coloration has been documented in many animals and is attributed to the synthesis of pteridines and acquisition of carotenoids from the diet (Grether, Hudon, & Endler, 2001; Hill, Inouye, & Montgomerie, 2002; Sefc, Brown, & Clotfelter, 2014). For *P. dorsalis*, however, dietary overlap between urban and rural populations is high (80%) and the only notable difference is in the number of ants consumed (Balakrishna et al., 2016), which are not likely to contribute to yellow-red pigmentation. Thus, differences in the dorsal colour pattern between suburban and rural males of *P. dorsalis* are unlikely to be due to dietary differences. Ecological and social factors in the anthropogenically disturbed and natural habitats, however, can affect coloration in animals. Unlike rural habitats, urban areas are more heterogeneous in a range of ways, from more varied substrate types and different microhabitat conditions (Gomez & Théry, 2004; Leal & Fleishman, 2003) to higher unpredictability and greater changes in habitat structure (Gilbert, 1991). In our study area, habitat fragmentation has resulted in higher local densities in the suburban population (Amdekar & Thaker, 2017), potentially habituating males to repeated close-range social interactions. Thus, low colour contrasts in suburban males might reflect a reduction in the need to signal intensively in high-density social conditions. Composition and abundance of predator communities might also be different in suburban than rural areas (e.g. Amdekar & Thaker, 2017; Thorington & Bowman, 2003). A combination of variable microhabitat conditions, changes in local densities and differences in predation pressure may interact to influence the intensity of visual signals, potentially shifting communication strategies of *P. dorsalis* in these remnant urbanized habitats.

Acknowledgments

We thank S. Balakrishna for help in the field, D. Stuart-Fox for advice on visual modelling and J. Endler for MATLAB scripts used in colour linearization. We also thank D. Stuart-Fox, A.T. Vanak, and two anonymous referees for helpful comments on the manuscript. The authors have no competing interests. This work was supported by the DBT-IISc partnership program, and the Ministry of Environment, Forests and Climate Change. University Grants Commission supported the research fellowship for A.B.

References

- Amdekar, M., & Thaker, M. (2017). *Variable riskiness of sexual colours across an urban-rural landscape* (Manuscript in preparation).
- Andersson, S., & Andersson, M. (1998). Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proceedings of the Royal Society B: Biological Sciences*, 265(1395), 445–450.
- Anibaldi, C., Luiselli, L., & Angelici, F. M. (1998). Notes on the ecology of a suburban population of rainbow lizards in coastal Kenya. *African Journal of Ecology*, 36(3), 199–206.
- Balakrishna, S., Batabyal, A., & Thaker, M. (2016). Dining in the city: Dietary shifts in Indian rock agamas across an urban – rural landscape. *Journal of Herpetology*, 50(3), 423–428.
- Barbour, H. R., Archer, M. A., Hart, N. S., Thomas, N., Dunlop, S. A., Beazley, L. D., et al. (2002). Retinal characteristics of the ornate dragon lizard, *Ctenophorus ornatus*. *Journal of Comparative Neurology*, 450(4), 334–344.
- Bateman, P. W., & Fleming, P. A. (2014). Does human pedestrian behaviour influence risk assessment in a successful mammal urban adapter? *Journal of Zoology*, 294(2), 93–98.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. R Package Version, 1(7). <http://cran.r-project.org/package=lme4>.
- Boback, S. M., & Siefferman, L. M. (2010). Variation in color and color change in island and mainland boas (*Boa constrictor*). *Journal of Herpetology*, 44(4), 506–515.

- Brown, C., Garwood, M. P., & Williamson, J. E. (2012). It pays to cheat: Tactical deception in a cephalopod social signalling system. *Biology Letters*, rsbl20120435.
- Camargo, C. R., Visconti, M. A., & Castrucci, A. M. L. (1999). Physiological color change in the bullfrog, *Rana catesbeiana*. *Journal of Experimental Zoology A: Ecological Genetics and Physiology*, 283(2), 160–169.
- Candolin, U. (2000). Male-male competition ensures honest signaling of male parental ability in the three-spined stickleback (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology*, 49(1), 57–61.
- Clarke, J. M., & Schluter, D. (2011). Colour plasticity and background matching in a three spine stickleback species pair. *Biological Journal of the Linnean Society*, 102(4), 902–914.
- Cole, G. L., & Endler, J. A. (2015). Variable environmental effects on a multicomponent sexually selected trait. *American Naturalist*, 185(4), 452–468.
- Cooper, W. E., & Greenberg, N. (1992). Reptilian coloration and behavior. In C. Gans, & D. Crews (Eds.), *Biology of the Reptilia* (pp. 298–422). Chicago: IL: University of Chicago Press.
- Dale, J. (2006). Intraspecific variation in coloration. In K. J. McGraw (Ed.), *Bird coloration* (pp. 36–86). Harvard, MA: Harvard University Press.
- Deodhar, S. (2017). *Sexual selection and personality in Psammophilus dorsalis* (Doctoral dissertation). Bangalore, India: Indian Institute of Science.
- Dubuc, C., Allen, W. L., Maestripieri, D., & Higham, J. P. (2014). Is male rhesus macaque red color ornamentation attractive to females? *Behavioral Ecology and Sociobiology*, 68(7), 1215–1224.
- Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research*, 31(3), 587–608.
- Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *American Naturalist*, 139, 125–153.
- French, S. S., Fokidis, H. B., & Moore, M. C. (2008). Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban–rural gradient. *Journal of Comparative Physiology B*, 178(8), 997–1005.
- Fukuda, S., & Karino, K. (2014). Male red coloration, female mate preference, and sperm longevity in the cyprinid fish *Puntius titteya*. *Environmental Biology of Fishes*, 97(11), 1197–1205.
- Gilbert, O. (1991). *The ecology of urban habitats*. New York, NY: Springer Science & Business Media.
- Gomez, D., & Théry, M. (2004). Influence of ambient light on the evolution of colour signals: Comparative analysis of a neotropical rainforest bird community. *Ecology Letters*, 7(4), 279–284.
- Grether, G. F., Hudon, J., & Endler, J. A. (2001). Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B: Biological Sciences*, 268(1473), 1245–1253.
- Hamilton, P. S., & Sullivan, B. K. (2005). Female mate attraction in ornate tree lizards, *Urosaurus ornatus*: A multivariate analysis. *Animal Behaviour*, 69(1), 219–224.
- Hamilton, D. G., Whiting, M. J., & Pryke, S. R. (2013). Fiery frills: Carotenoid-based coloration predicts contest success in frillneck lizards. *Behavioral Ecology*, 24(5), 1138–1149.
- Hamilton, W. D., & Zuk, M. (1982). Heritable true fitness and bright birds: A role for parasites? *Science*, 218(4570), 384–387.
- Hanlon, R. (2007). Cephalopod dynamic camouflage. *Current Biology*, 17(11), 400–404.
- Healey, M., Uller, T., & Olsson, M. (2007). Seeing red: Morph-specific contest success and survival rates in a colour-polymorphic agamid lizard. *Animal Behaviour*, 74(2), 337–341.
- Hebets, E. A., & Papaj, D. R. (2005). Complex signal function: Developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology*, 57(3), 197–214.
- Hemmi, J. M., Marshall, J., Pix, W., Vorobyev, M., & Zeil, J. (2006). The variable colours of the fiddler crab *Uca vomeris* and their relation to background and predation. *Journal of Experimental Biology*, 209(20), 4140–4153.
- Hill, G. E. (2006). Female mate choice for ornamental coloration. *Bird Coloration*, 2, 137–200.
- Hill, G. E., Inouye, C. Y., & Montgomerie, R. (2002). Dietary carotenoids predict plumage coloration in wild house finches. *Proceedings of the Royal Society B: Biological Sciences*, 269(1496), 1119–1124.
- Hothorn, T., Bretz, F., & Westfal, P. (2013). Package 'multcomp': Simultaneous inference in general parametric models. *R Package Version*, 1.2–13. cran.r-project.org/web/packages/multcomp/multcomp.pdf.
- Hutton, P., & McGraw, K. J. (2016). Urban Impacts on oxidative balance and animal signals. *Frontiers in Ecology and Evolution*, 4, 54.
- Inoué, S., & Inoué, Z. (1977). Colour changes induced by pairing and painting in the male rainbow lizard, *Agama agama*. *Cellular and Molecular Life Sciences*, 33(11), 1443–1444.
- Jones, T. M., Rodewald, A. D., & Shustack, D. P. (2010). Variation in plumage coloration of Northern Cardinals in urbanizing landscapes. *Wilson Journal of Ornithology*, 122(2), 326–333.
- Kelley, J. L., Phillips, B., Cummins, G. H., & Shand, J. (2012). Changes in the visual environment affect colour signal brightness and shoaling behaviour in a freshwater fish. *Animal Behaviour*, 83(3), 783–791.
- Kempnaers, B., Borgström, P., Loës, P., Schlicht, E., & Valcu, M. (2010). Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Current Biology*, 20(19), 1735–1739.
- Keren-Rotem, T., Levy, N., Wolf, L., Bouskila, A., & Geffen, E. (2016). Male preference for sexual signalling over crypsis is associated with alternative mating tactics. *Animal Behaviour*, 117, 43–49.
- Kindermann, C., Narayan, E. J., Wild, F., Wild, C. H., & Hero, J. M. (2013). The effect of stress and stress hormones on dynamic colour-change in a sexually dichromatic Australian frog. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 165(2), 223–227.
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2015). Package 'lmerTest'. *R package version 2*. cran.r-project.org/package=lmerTest.
- Langkilde, T., & Boronow, K. E. (2012). Hot boys are blue: Temperature-dependent color change in male eastern fence lizards. *Journal of Herpetology*, 46(4), 461–465.
- Lazić, M. M., Kaliontzopoulou, A., Carretero, M. A., & Crnobrnja-Isailović, J. (2013). Lizards from urban areas are more asymmetric: Using fluctuating asymmetry to evaluate environmental disturbance. *PLoS One*, 8(12), e84190.
- Leal, M., & Fleishman, L. J. (2003). Differences in visual signal design and detectability between allopatric populations of Anolis lizards. *American Naturalist*, 163(1), 26–39.
- LeBas, N. R., & Marshall, N. J. (2000). The role of colour in signalling and male choice in the agamid lizard *Ctenophorus ornatus*. *Proceedings of the Royal Society B: Biological Sciences*, 267(1442), 445–452.
- Ligon, R. A., & McGraw, K. J. (2013). Chameleons communicate with complex colour changes during contests: Different body regions convey different information. *Biology Letters*, 9(6), 20130892.
- Longcore, T., & Rich, C. (2004). Ecological light pollution. *Frontiers in Ecology and the Environment*, 2(4), 191–198.
- Marshall, K. L., Philpot, K. E., Damas-Moreira, I., & Stevens, M. (2015). Intraspecific colour variation among lizards in distinct island environments enhances local camouflage. *PLoS One*, 10(9), e0135241.
- Messenger, J. B. (2001). Cephalopod chromatophores: Neurobiology and natural history. *Biological Reviews*, 76(4), 473–528.
- Morris, M. R., Mussel, M., & Ryan, M. J. (1995). Vertical bars on male *Xiphophorus multilineatus*: A signal that deters rival males and attracts females. *Behavioral Ecology*, 6(3), 274–279.
- Nemeth, E., Pieretti, N., Zollinger, S. A., Geberzahn, N., Partecke, J., Miranda, A. C., et al. (2013). Bird song and anthropogenic noise: Vocal constraints may explain why birds sing higher-frequency songs in cities. *Proceedings of the Royal Society B: Biological Sciences*, 280(1754), 20122798.
- Norfolk, O., Melotte, A., Gilbert, F., Zalat, S., & Reader, T. (2010). A comparative study of two agamid lizards, *Laudakia stellio* and *Pseudotrapelus sinaitus*, in southern Sinai. *Egyptian Journal of Biology*, 12(1), 27–43.
- Partan, S. R., & Marler, P. (2005). Issues in the classification of multimodal communication signals. *American Naturalist*, 166(2), 231–245.
- Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos*, 118(12), 1883–1891.
- R Core Team. (2016). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. www.R-project.org.
- Radder, R. S., & Saidapur, S. K. (2005). Population density, microhabitat use and activity pattern of the Indian rock lizard, *Psammophilus dorsalis* (Agamidae). *Current Science*, 89(3), 275–283.
- Radder, R. S., Saidapur, S. K., Shine, R., & Shanbhag, B. A. (2006). The language of lizards: Interpreting the function of visual displays of the Indian rock lizard, *Psammophilus dorsalis* (Agamidae). *Journal of Ethology*, 24(3), 275–283.
- Rankin, K., & Stuart-Fox, D. (2015). Testosterone-induced expression of male colour morphs in females of the polymorphic tawny dragon lizard, *Ctenophorus decresii*. *PLoS One*, 10(10), e0140458.
- Rosenthal, G. G., & Stuart-Fox, D. (2012). Environmental disturbance and animal communication. In B. M. Wong, & U. Candolin (Eds.), *Behavioural responses to a changing world: mechanisms and consequences* (pp. 16–31). Oxford, U.K.: Oxford University Press.
- Sefc, K. M., Brown, A. C., & Clotfelter, E. D. (2014). Carotenoid-based coloration in cichlid fishes. *Comparative Biochemistry and Physiology Part a: Molecular & Integrative Physiology*, 173, 42–51.
- Smith, K. R., Cadena, V., Endler, J. A., Porter, W. P., Kearney, M. R., & Stuart-Fox, D. (2016). Colour change on different body regions provides thermal and signalling advantages in bearded dragon lizards. *Proceedings of the Royal Society B: Biological Sciences*, 283(1832), 20160626.
- Sol, D., Lapidra, O., & González-Lagos, C. (2013). Behavioural adjustments for a life in the city. *Animal Behaviour*, 85(5), 1101–1112.
- Srinivas, S. R., Hegde, S. N., Sarkar, H. B. D., & Shivanandappa, T. (1995). Sperm storage in the oviduct of the tropical rock lizard, *Psammophilus dorsalis*. *Journal of Morphology*, 224(3), 293–301.
- Stevens, M., Parraga, C. A., Cuthill, I. C., Partridge, J. C., & Troscianko, T. S. (2007). Using digital photography to study animal coloration. *Biological Journal of the Linnean Society*, 90(2), 211–237.
- Stuart-Fox, D. M., & Johnston, G. R. (2005). Experience overrides colour in lizard contests. *Behaviour*, 142(3), 329–350.
- Stuart-Fox, D., & Moussalli, A. (2008). Selection for social signalling drives the evolution of chameleon colour change. *PLoS Biology*, 6(1), e25.
- Stuart-Fox, D., & Moussalli, A. (2009). Camouflage, communication and thermoregulation: Lessons from colour changing organisms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1516), 463–470.
- Teasdale, L. C., Stevens, M., & Stuart-Fox, D. (2013). Discrete colour polymorphism in the tawny dragon lizard (*Ctenophorus decresii*) and differences in signal

- conspicuousness among morphs. *Journal of Evolutionary Biology*, 26(5), 1035–1046.
- Teyssier, J., Saenko, S. V., Van Der Marel, D., & Milinkovitch, M. C. (2015). Photonic crystals cause active colour change in chameleons. *Nature Communications*, 6, 6368.
- Thorington, K. K., & Bowman, R. (2003). Predation rate on artificial nests increases with human housing density in suburban habitats. *Ecography*, 26(2), 188–196.
- Tringali, A., & Bowman, R. (2015). Suburban immigrants to wildlands disrupt honest signaling in ultra-violet plumage. *Avian Conservation and Ecology*, 10(1), 9.
- Tuomainen, U., & Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biological Reviews*, 86(3), 640–657.
- White, T. E., Dalrymple, R. L., Noble, D. W., O'Hanlon, J. C., Zurek, D. B., & Umbers, K. D. (2015). Reproducible research in the study of biological coloration. *Animal Behaviour*, 106, 51–57.
- Wong, B. B., & Candolin, U. (2015). Behavioral responses to changing environments. *Behavioral Ecology*, 26(3), 665–673.
- Wong, B. B., Candolin, U., & Lindström, K. (2007). Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. *American Naturalist*, 170(2), 184–189.
- Wood, W. E., & Yezerinac, S. M. (2006). Song sparrow (*Melospiza melodia*) song varies with urban noise. *Auk*, 123(3), 650–659.
- Yewers, M. S., McLean, C. A., Moussalli, A., Stuart-Fox, D., Bennett, A. T., & Knott, B. (2015). Spectral sensitivity of cone photoreceptors and opsin expression in two colour-divergent lineages of the lizard *Ctenophorus decresii*. *Journal of Experimental Biology*, 218(10), 1556–1563.
- Zylinski, S., How, M. J., Osorio, D., Hanlon, R. T., & Marshall, N. J. (2011). To be seen or to hide: Visual characteristics of body patterns for camouflage and communication in the Australian giant cuttlefish *Sepia apama*. *American Naturalist*, 177(5), 681–690.