



Original Article

Flies improve the salience of iridescent sexual signals by orienting toward the sun

Thomas E. White[✉] and Tanya Latty

School of Life and Environmental Sciences, The University of Sydney, Camperdown, Sydney, New South Wales 2106, Australia

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Sunlight is the ultimate source of most visual signals. Theory predicts strong selection for its effective use during communication, with functional links between signal designs and display behaviors a likely result. This is particularly true for iridescent structural colors, whose moment-to-moment appearance bears a heightened sensitivity to the position of signalers, receivers, and the sun. Here, we experimentally tested this prediction using *Lispe cana*, a muscid fly in which males present their structurally colored faces and wings to females during ground-based sexual displays. In field-based assays, we found that males actively bias the orientation of their displays toward the solar azimuth under conditions of full sunlight and do so across the entire day. This bias breaks down, however, when the sun is naturally concealed by heavy cloud or experimentally obscured. Our modeling of the appearance of male signals revealed clear benefits for the salience of male ornaments, with a roughly 4-fold increase in subjective luminance achievable through accurate display orientation. These findings offer fine-scale, causal evidence for the active control of sexual displays to enhance the appearance of iridescent signals. More broadly, they speak to predicted coevolution between dynamic signal designs and presentation behaviors, and support arguments for a richer appreciation of the fluidity of visual communication.

Key words: sensory drive, sexual selection, structural coloration, wing interference pattern.

INTRODUCTION

The structure of a communication system is shaped by its response to basic challenges. Encoding information (Weaver et al. 2018; White 2020), ensuring its delivery (Endler and Thery 1996), and separating signal from noise (Warrant 2016) are essential features of signaling systems, and selection has generated diverse solutions. In general terms, theory predicts the coevolution of signals and signaling behaviors that secure the exchange of information between interested parties while minimizing exposure to eavesdroppers (à la “sensory drive”; Endler 1992, 1993). Males of the common eggfly *Hypolimnas bolina*, for example, combine limited-view iridescent colors with precision display behaviors to deliver maximally conspicuous signals during courtship (White et al. 2015), and females prefer males bearing the most striking ornamentation (Kemp 2007). Great bowerbirds, by contrast, present colored objects to females from within constructed viewing “theatres,” and in doing so control both the object of display and the broader visual environment (Endler et al. 2010; Kelley and Endler 2012).

The composition of sunlight shapes the appearance of most visual signals, as its color and intensity—following any modification during transmission—shape the appearance of reflective surfaces. There should therefore be intense selection for its effective

use during communication (Endler 1992, 1993). This is particularly true for signal designs whose moment-to-moment appearance bears a heightened sensitivity to local conditions, such as structural colors (Kinoshita 2008; Mouchet and Vukusic 2018). Structural colors result from the selective reflectance of light by tissues organized at the nanoscale and can achieve levels of brightness and chromaticity that are unattainable with pigments alone (Kinoshita 2008; Johnsen 2012). Where coherent or quasi-coherent scattering is involved, as is most often the case, they are also capable of iridescence and limited-view expression (Prum 2006; Vukusic 2006).

Given that the optimal color and/or brightness of iridescent signals is tied to the geometry of the signaler, viewer, and illuminant, its widespread use in nature suggests the existence of flexible behaviors for their effective presentation. That is, signalers should seek to capture available sunlight so as to maximize the conspicuousness of structurally colored signals to viewers (Endler 1992; Endler and Thery 1996). This prediction has found broad support across model vertebrates, with observational evidence showing how signalers vary the timing, location, or orientation of their displays to improve communication efficacy (Endler 1991; Endler and Thery 1996; Dakin and Montgomerie 2009; Bortolotti et al. 2011; Sicsu et al. 2013; Klomp et al. 2017; Simpson and McGraw 2018; O’Neill et al. 2019). Though this work is extremely valuable, a richer causal understanding of the precision, mechanisms of control, and adaptive value of such presentation behaviors awaits manipulative tests across broader temporal and taxonomic contexts. This is

Address correspondence to T. E. White. E-mail: thomas.white@sydney.edu.au.

essential if we are to understand the generality of predicted solutions to shared challenges in communication (Endler 1992; Kelley 2006).

Flies rank among the most diverse animal orders and this is reflected in the traits that have evolved in the service of visual communication (Marshall 2012). Sexually and ecologically specialized eye structures (Zeil 1983; Hardie 1986), elaborate color patterns (Shevtsova et al. 2011; Butterworth et al. 2020), and ritualized display behaviors (Land 1993; Zimmer et al. 2003; Lunau et al. 2006; Butterworth et al. 2019) abound, and offer tractable but underutilized opportunities for general tests of theory. Work on visual signaling in the group continues to document the widespread presence of conspicuous structural colors, with recent efforts highlighting “wing interference patterns” (WIPs) as potential vectors of information (Shevtsova et al. 2011; Katayama et al. 2014; Hawkes et al. 2019; Butterworth et al. 2020). These striking patterns are the result of thin film interference from chitin/air interfaces in the wing membrane (Shevtsova et al. 2011) and they are regularly presented during courtship (Land 1993; Frantsevich and Gorb 2006; Katayama et al. 2014; White et al. 2020). The wings bearing them are also typically transparent and transmit ca. 70–80% of incident light. This combination of limited-view iridescence and transparency makes their appearance uniquely environmentally contingent since bright visual backgrounds or the misalignment of signalers, receivers, and the sun will render the patterns invisible (see Fleishman et al. 2006, 2016, for discussion of related effects in a transparent, but non-iridescent, signal). The significance of visual backgrounds during signaling is well demonstrated (Katayama et al. 2014) and includes evidence for active choice in the wild (White et al. 2020). Whether and how signalers actively use the sun to improve communication, however, is unknown.

The genus *Lispe* is a cursorial group of muscid flies comprised at least 163 species spanning most terrestrial biogeographic regions (Pont 2019). The group showcases striking interspecific and sexual variation in coloration and displays (Frantsevich and Gorb 2006; Pont 2019). *Lispe cana* is an exemplar of the genus endemic to the foreshores of Australia’s east coast. Males and females of the species bear structurally colored UV-white and yellow faces, respectively, as well as sexually dimorphic WIPs (White TE, unpublished data). They live in relatively high densities (ca. 5–65 individuals m² in preferred habitat), and courtships occur continuously throughout the day across a breadth of environmental conditions (Pont 2019; though their encounter rates vary under weather extremes—see

Results). During their ground-based courtship, male *L. cana* approach and straddle females from behind and maintain this position as females walk about the environment. They then release and move directly in front of females to present their faces and wings in a ritualized “dance.” Males are able to enhance the salience of their signals through the selection of backgrounds against which to display, and similar potential benefits exist through the careful use of sunlight (White et al. 2020).

Here, we use observational and manipulative field-based assays at a fine temporal scale to test the hypothesis that males should orient their displays toward the sun so to improve the salience (and, hence, the likely attractiveness; White et al. 2020) of their iridescent signals. This predicts a bias in the orientation of male displays toward the sun’s azimuth under clear skies, when its position is readily visible and the potential benefits for signal salience are likely maximized. As a corollary, the natural or artificial removal of direct sunlight should disrupt any orientation bias by either impeding a male’s ability to orient and/or diminishing the benefits of such behavior for the appearance of signals.

METHODS

Sexual displays in *Lispe cana*

Courtship in *L. cana* proceeds in the following four general stages (Figure 1): (1) males detect females when both parties are walking about their foreshore habitats; (2) males approach females from behind, before “straddling” them and holding their wings closed as they continue to walk about; (3) males release females and rapidly move immediately in front of them at close distance (<5 mm), where they present their colorful wings and faces as part of a ritualized display; (4) either the sequence returns to (2) with male re-straddling females, they mate, or (most often) they disperse. The second stage—straddling—thus offers males the opportunity to assess the environment and their orientation within it, while the third stage is when their decision to display at a given location and orientation is realized and is therefore the focus of our observations.

Behavioral observations and assays

We observed courtships in a population of *L. cana* that inhabits the supralittoral zone of Toowoomb Bay, NSW, Australia (33.36 S, 151.50 E), in September and October of 2019. During each

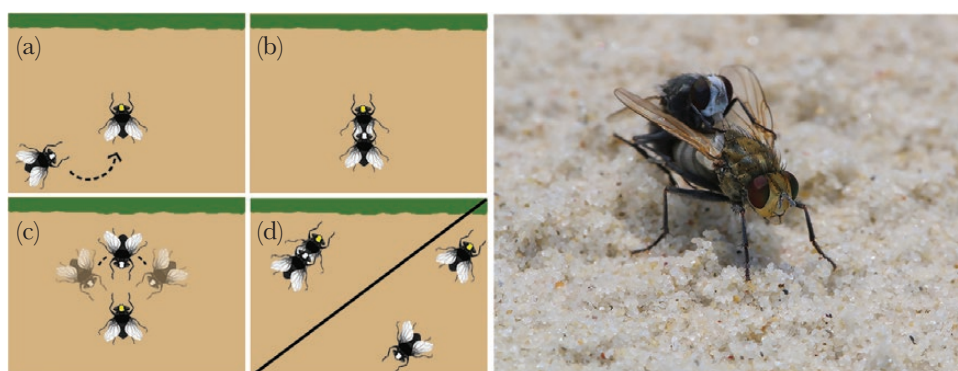


Figure 1

Courtship in the muscid fly *Lispe cana* (right). (a) Males (white face) first detect females (gold face), before rapidly approaching and (b) straddling them while holding their wings closed as they walk through their foreshore habitats. Males then release and move in front of females to display (c), during which they present their structurally colored wings and faces in a ritualized “dance.” (d) Courtship ends with mating, a return to straddling or, most often, the dispersal of both parties. Panels (a)–(d) modified from White et al. 2020 (photograph: Nathan J. Butterworth).

courtship, we recorded the orientation of the male during the display phase (stage 3; Figure 1) of their ritualized dance, which we observed while standing stationary at a distance of 1–2 m. The presence of an observer seemed to cause no disturbance, which is supported by the correlation between measurements taken live and via videos captured at greater working distances. Courting pairs were haphazardly selected during slow, repeated walks by an observer along a 255 m transect at a distance of 5–10 m from the shoreline. Once a courtship naturally terminated with both parties dispersing, we placed a straight marker at the males' final position in alignment with their medial body axis, before recording the markers' orientation to the nearest one degree using a digital compass, as well as the time. To initially validate the accuracy of this approach, we tested it against a video-based method in which male orientations were extracted from still-frames taken from 25 video-recorded courtships (with a GoPro Hero 6 at 30fps) in which a compass was visible in-frame. We found a strong correlation between orientations collected from video versus direct observation (Pearson's $r = 0.88$), so we favored the latter method for its convenience in garnering a large sample size. We did not track individual flies between courtships which raises the possibility of minor pseudo-replication. However, the tendency of flies to disperse after courtship, their high population density, and our broad spatial and temporal sampling regime together imply a low probability of any single individual contributing to multiple sequences.

Once courtships were observed, we used the time and location data to calculate the solar azimuth and elevation during every courtship via the package “suncalc” (v. 0.5.0; Thieurmel and Elmarhraoui 2019) for R (v. 3.6.0; R Core Team 2018). We then converted our circular measure of male display orientations to a linear measure of the absolute deviation of each display from the sun's azimuth. This gives a value between 0 and 180°, with 0° being a male displaying exactly toward sun, and 180° directly away from it. We ignore the position of females because males actively position themselves approximately opposite females at < 5 mm distance during displays (Figure 1).

To test the extent to which males can and do orient their displays, we used the general procedure above across one manipulative and two observational conditions. In the two observational conditions, we recorded displays between the hours of 0730 and 1630 either on days of full direct sunlight ($n = 451$ observations across 6 days), or heavy cloud with the sun completely obscured ($n = 227$ across 3 days). For the manipulative assay, we repeated the procedure as for full sun conditions (including time period and general location) but obscured any direct sunlight using a static black plastic sheet (Monarch pty. ltd) suspended on four aluminum poles ca. 10 m from the shoreline. This cast a ca. 3×3 m shadow throughout a given day, and we recorded only courtships that began and finished within this shaded area ($n = 191$ across 3 days). The broader environmental conditions were similar across days within treatments (summarized in Supplementary Table S1) and are partially accounted for in our statistical analyses.

Solar orientation and signal luminance

To model the effects of male orientation on the salience of their signals, we first recorded vector irradiances at the study site in 10° increments from 0° (directly toward the sun) to 180° (directly away) at 0800, under representative conditions of full sunlight and heavy cloud. In both cases, we used the online version of “suncalc” (Thieurmel and Elmarhraoui 2019) to zero our initial

(0°) orientation toward the sun's precise azimuth. We collected all spectra using an OceanInsight JAZ spectrophotometer with cosine-corrector and oriented the 180° collector perpendicular to the ground (i.e., in the same plane as male faces and wings during display). We then recorded the facial and whole-wing reflectance of 50 male *L. cana* using the JAZ with its pulsed-xenon light source, at an integration time of 40 ms and a boxcar width of 2. We used a bifurcated probe to both deliver and collect light, which we oriented normal to the face and wing planes at a working distances of ca. 5 mm. A 99% diffuse reflector (Labsphere, North Sutton, New Hampshire) and black velvet served as our light and dark standards, respectively, and we recalibrated the spectrometer between each measurement. These spectra were lightly LOESS smoothed before being averaged to generate a representative male facial and whole-wing reflectance spectrum (Supplementary Figure S1).

We then estimated how the luminance of male wings and faces varies as a function of male orientation toward the sun. Here and throughout, we focus on luminance alone given its importance over chromaticity (hue and saturation) for mating (White et al. 2020). To do so, we calculated the integrated product of solar irradiance at each 10° increment from the solar azimuth, male facial or wing reflectance, and the Rh1-6 “achromatic” receptor absorbance of *Musca domestica* (as the nearest available analogue to *L. cana*). We conducted all spectral processing and visual modeling using `lightr` v1.1 (Gruson, White, et al. 2019) and `pavo` v2.4.0 (Maia et al. 2019) for R v3.6.1 (R Core Team 2018).

Statistical analyses

To estimate whether male *L. cana* bias the orientation of their sexual displays toward the sun, we used a generalized linear mixed-effect model fit by restricted maximum-likelihood, with Gaussian error and an identity link function. We specified male deviation from the solar azimuth (0–180) as the response which we square-root transformed. We did so after our inspection of errors from an untransformed model suggested slightly non-normal and heteroscedastic residuals, which were improved to within acceptable margins (as visually assessed) following transformation.

We specified the interaction between experimental condition (sun, cloud, and sun-blocked) and solar elevation as a fixed effect, along with the main effects of each. If males do actively orient their displays the result should be a negative effect of the full sunlight condition on male display offset (i.e., a tendency toward smaller offsets from the solar azimuth in full sun), when compared with the cloud and sun-blocked conditions. Our inclusion of solar elevation tests for reduced display accuracy at higher solar elevations when the sun's azimuth is less readily discerned. Such an effect would only manifest under conditions of full sunlight when any display-orienting takes place, hence its inclusion via an interaction term. We specified the Julian date on which each measurement was taken as a random effect to account for variation associated with unmeasured within-day variables, such as broader climatic conditions (summarized in Supplementary Table S1 and included in the raw data).

To estimate the effects of male display orientation on signal luminance, we first ran generalized additive models (GAM) fit by restricted maximum-likelihood, with the offset from solar azimuth (in 10° increments across the 180° range) as the lone predictor of signal luminance. The four resulting models describe the possible variation in male facial and wing luminance as a function of their sexual display orientation, during conditions of full sun and heavy

cloud. We then used the results of the full-sun GAM alone to predict the luminance of male signals at their actual recorded orientations in both full-sun and heavy cloud conditions. For example, the facial luminance of a male who oriented their display at 5° offset from the sun would be estimated at ca. $28 \mu\text{mol}/\text{m}^2$, while the luminance of a male face oriented at 160° would be ca. $5 \mu\text{mol}/\text{m}^2$ (Figure 5c, red line). We used only the full-sun GAM after finding no relationship between signal luminance and orientation under heavy cloud (Figure 3d), and thus no possible benefits for signal salience to orienting toward the sun in such conditions (see Results). We then used Kruskal–Wallis rank sum tests, with η^2 as an effect size, to estimate the difference in achieved luminance between male displaying under full sun and heavy cloud, with their luminance predicted from the full-sun GAM. This, in effect, answers the question of what males achieve by orienting their displays under ideal conditions.

RESULTS

We found clear differences in the orientation of male sexual displays across conditions (Figures 2 and 3). A likelihood-ratio test on the overall model identified a significant effect of experimental treatment alone ($\chi^2 = 86.86$, $df = 2$, $P < 0.001$), with no evidence for an effect of solar elevation ($\chi^2 = 2.54$, $df = 1$, $P = 0.111$), nor any interaction between treatment and solar elevation (Figure 4; $\chi^2 = 0.81$, $df = 2$, $P = 0.665$). Individual estimates (Table 1) show this was chiefly driven by a bias toward smaller angular offsets by displaying

males under full-sun conditions alone (estimate = -3.11 ± 1.136 , $df = 9$, $t = -2.74$, $P = 0.02$). Male displays were oriented toward the sun at a median offset of $44^\circ \pm 38$ SD on days in which it was fully visible. However, this effect was absent in conditions of heavy cloud ($87^\circ \pm 50$ SD) and artificially obstructed sunlight ($96^\circ \pm 52$ SD), which did not statistically differ from one another.

Our GAMs showed that the luminance of male wings ($F_{7,94} = 356$, $P < 0.001$) and faces ($F_{7,94} = 355.50$, $P < 0.001$) follow a near-identical sigmoidal relationship with solar orientation when illuminated in full sunlight (Figure 5c). They are maximally luminant at 0° from the solar azimuth, inflect at ca. 45° (95% confidence interval [CI] = 37 – 53° faces, 43 – 47° wings), before reaching their minima at ca. 80° . The luminance of wings, as compared to faces, varied across a slightly compressed range owing to their reduced reflectance (Supplementary Figure S1), but was otherwise strongly correlated with facial luminance. This effect was entirely absent in conditions of heavy cloud (Figure 5d), as we found no relationship between either of facial ($F_1 = 0.190$, $P = 0.669$) or wing ($F_1 = 0.192$, $P = 0.667$) luminance and solar orientation.

Our subsequent test of signal luminance during actual male displays suggests clear benefits to active solar orientation in full sun. Both their faces ($\chi^2 = 86.97$, $df = 1$, $P < 0.01$, $\eta^2 = 0.127$) and wings ($\chi^2 = 86.70$, $df = 1$, $P < 0.01$, $\eta^2 = 0.126$) were subjectively brighter, with an approximately 4-fold increase as a result of their active display orienting. Given the absence of a relationship between signal luminance and solar orientation under heavy cloud, any active orientation by males could yield no benefits to signal salience in such conditions.

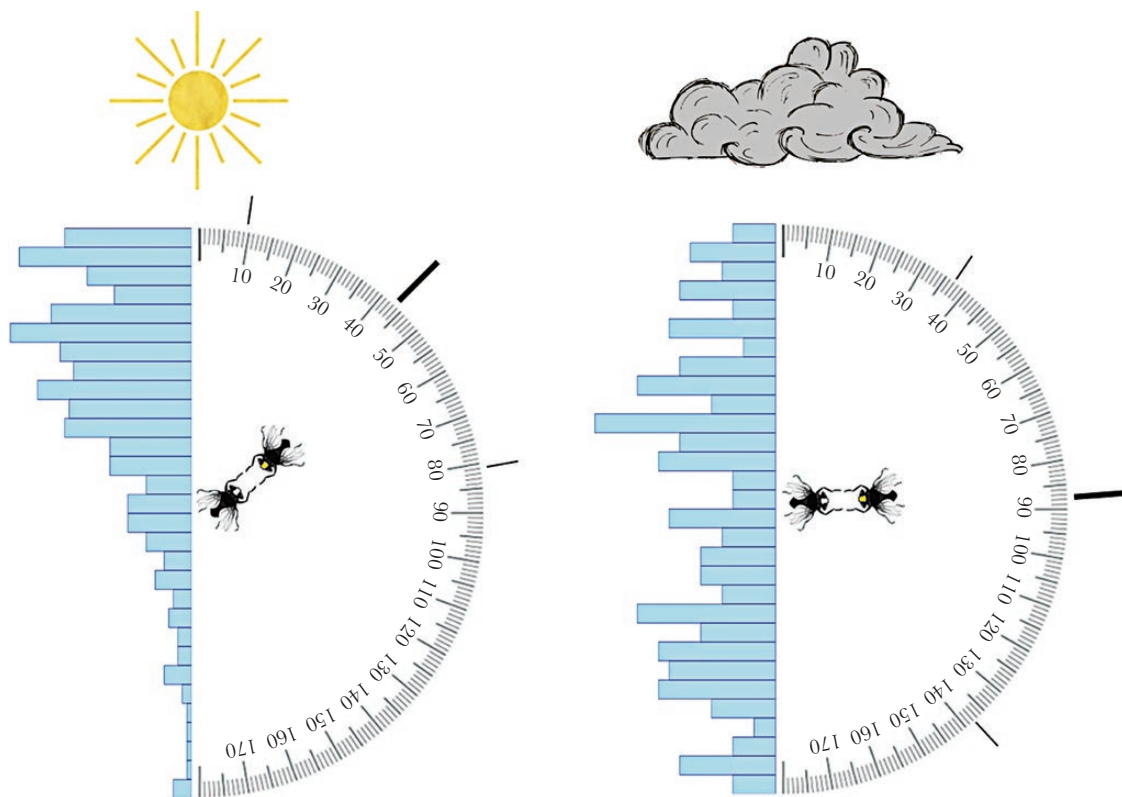


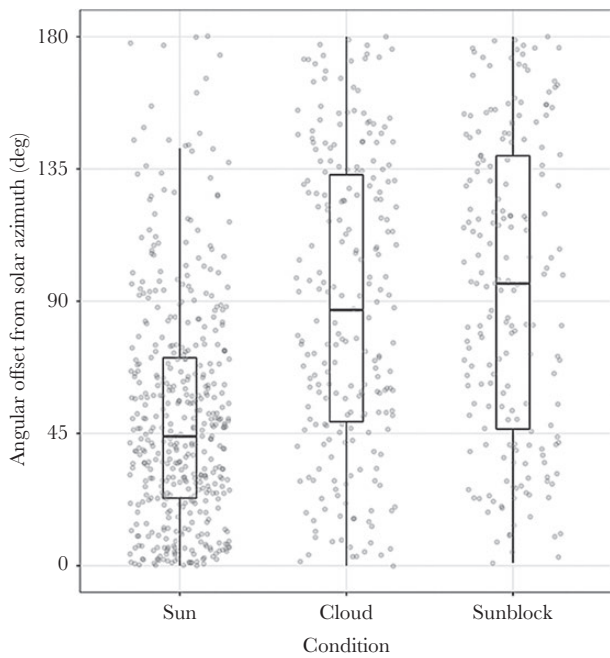
Figure 2

The orientations of *Lispe cana* during courtship displays under full sun (left) and heavy cloud (right). When displaying, male flies (white face) position themselves immediately in front of females (yellow face), and twist their wings forward in a seemingly ritualized “dance.” Angles denote the offset of the male midline from the solar azimuth during courtship, with 0° representing a display directly toward the sun, and 180° directly away. Histograms show the distribution of male offsets from the sun under both conditions ($n = 450$ full sun, $n = 227$ heavy cloud), with the median \pm standard deviation indicated by solid lines.

Table 1

Parameter estimates and summary statistics from a generalized linear mixed model examining the predictors of male display orientation in *Lispe cana*. Included are the experimental condition (full sun, heavy cloud, and artificially obscured sunlight) and solar elevation. Julian date was included as a random effect with a variance of 0.301. Full model conditional $R^2 = 0.169$

Fixed effects	Estimate	SE	df	<i>t</i>	<i>P</i>
Intercept	8.389	0.771	853	10.88	<0.001
Condition (sun)	-3.115	1.336	9	-2.743	0.023
Condition (sunblock)	0.425	1.014	9	0.419	0.685
Solar elevation	0.011	0.016	853	0.712	0.476
Condition (sun) × solar elevation	0.014	0.023	853	0.624	0.533
Condition (sunblock) × solar elevation	-0.003	0.020	853	-0.191	0.849

**Figure 3**

The orientation of displaying male *Lispe cana* with respect to the sun in conditions of full sun, heavy cloud, or experimentally obscured sunlight. Orientations are expressed as offsets from the solar azimuth, pooled across the 0730–1630 observation period, with 0° representing males presenting their iridescent signals directly toward the sun and 180° directly away. The null expectation is a uniform distribution of offsets, with a mean at approximately 90°.

DISCUSSION

The intensity and spectral composition of sunlight shapes the salience of visual signals, and selection should favor its flexible use. Here, we tested this prediction in the cursorial fly *L. cana* whose structurally colored faces and wings are central to their ritualized courtship displays. Our field-based observations show that males bias the orientation of their signals toward the sun under clear skies, with individuals displaying at approximately 44° offset from the solar azimuth on average. This degree of precision is maintained throughout the day irrespective of solar elevation. Notably, however, the directional bias in displays broke down in conditions of heavy cloud, and we were able to reconstruct this effect by experimentally obstructing the sun under otherwise-ideal conditions. Our visual modeling of signal luminance identified clear benefits to male display behavior, with an approximately 4-fold difference in

the fly-subjective luminance of male signals across the 180° range of possible orientations. These combined results offer causal evidence that male flies actively orient their conspicuous displays toward the sun, with tangible consequences for the salience of their structurally colored signals. Below, we consider our findings in turn and discuss their relevance for communication efficacy and signal evolution more broadly.

One immediate question is how male *L. cana* reliably orient their displays throughout the entire day. The sun is the brightest spot in the sky so tracking its position directly is an obvious solution, and this information can be supplemented through the use of indirect cues such as intensity, chromaticity, and polarization gradients. On clear days, the intensity of sunlight increases predictably toward the solar azimuth and atmospheric scattering means the sky is relatively richer in long-wavelength light in the solar, as opposed to anti-solar, hemisphere (Figure 5a; Coemans et al. 1994). The degree of polarization is similarly graded and increases with the angular distance from the sun (Coemans et al. 1994). The integrated use of such cues is well described among insects, including flies (Philipsborn et al. 1990; Weir and Dickinson 2012), albeit typically in the context of navigation. Monarch butterflies, for example, rely on direct and indirect skylight cues to calibrate their time-compensated sun compass during annual migrations (Heinze and Reppert 2011; Reppert et al. 2016), while desert ants integrate solar intensity, chromaticity, and polarization during navigation to and from the nest (Müller and Wehner 2007; Leubardt and Ronacher 2015).

Though our results cannot directly test whether male *L. cana* make use of all available cues when orienting, the suppression of their orientation bias with the obstruction of direct sunlight—both artificial and natural—suggests that they first and foremost rely on the sun's immediate position. This follows from the fact that gradients in each of polarization, and (to a lesser extent) luminance and chromaticity are present even when the sun is not directly visible, and many flies are able to use this information to guide behavior in other contexts (Philipsborn et al. 1990; Weir and Dickinson 2012). However, while there is a reason to expect that males *should* be able to orient their displays in nonoptimal conditions, our modeling suggests that there is no benefit in doing so. Signal luminance bears no relationship to solar orientation under heavy cloud (Figure 5d), so males should instead prioritize more consequential effects such as the selection of suitable display backgrounds (White et al. 2020). Whether they do so remains to be seen, and could be profitably tested in controlled conditions by manipulating the spectral composition of background material and orientation(s) of an artificial point-source illuminant.

The importance of accurate solar orientation during courtship is heightened by *L. cana*'s use of structurally colored ornaments. This

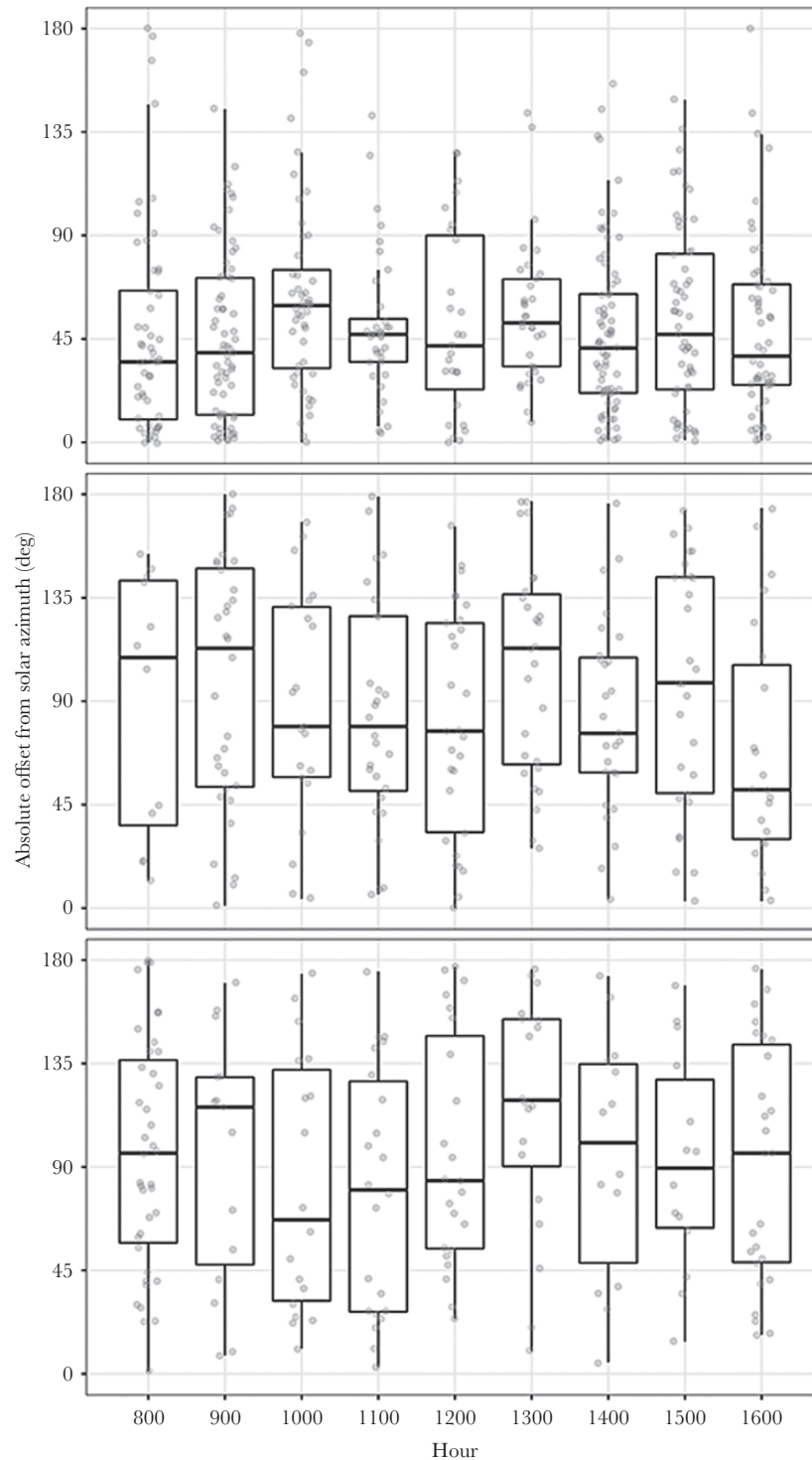


Figure 4

The orientation of displaying male *Lispe cana* in conditions of full sun (top), heavy cloud (middle), or experimentally obscured sunlight (bottom), pooled into hourly periods. Orientations are expressed as offsets from the solar azimuth, with 0° representing males presenting their iridescent signals directly toward the sun and 180° directly away. The null expectation is a relatively uniform distribution of offsets, with a mean at approximately 90° .

is particularly true for WIPs, the color and luminance of which is a product of thin-film interference from the layering of chitinous wing membranes (Shevtsova et al. 2011). Because these colors are highly specular (i.e., dominated by mirror-like reflection) in nature, the absence of a point-source illuminant such as the sun can render

the patterns indistinguishable (Shevtsova et al. 2011). Males that are less able to optimize their orientation would therefore suffer penalty not only in the quality of their wing coloration (e.g., via reduced intensity), but the “category” too, as the patterns will be entirely absent at oblique illumination angles.

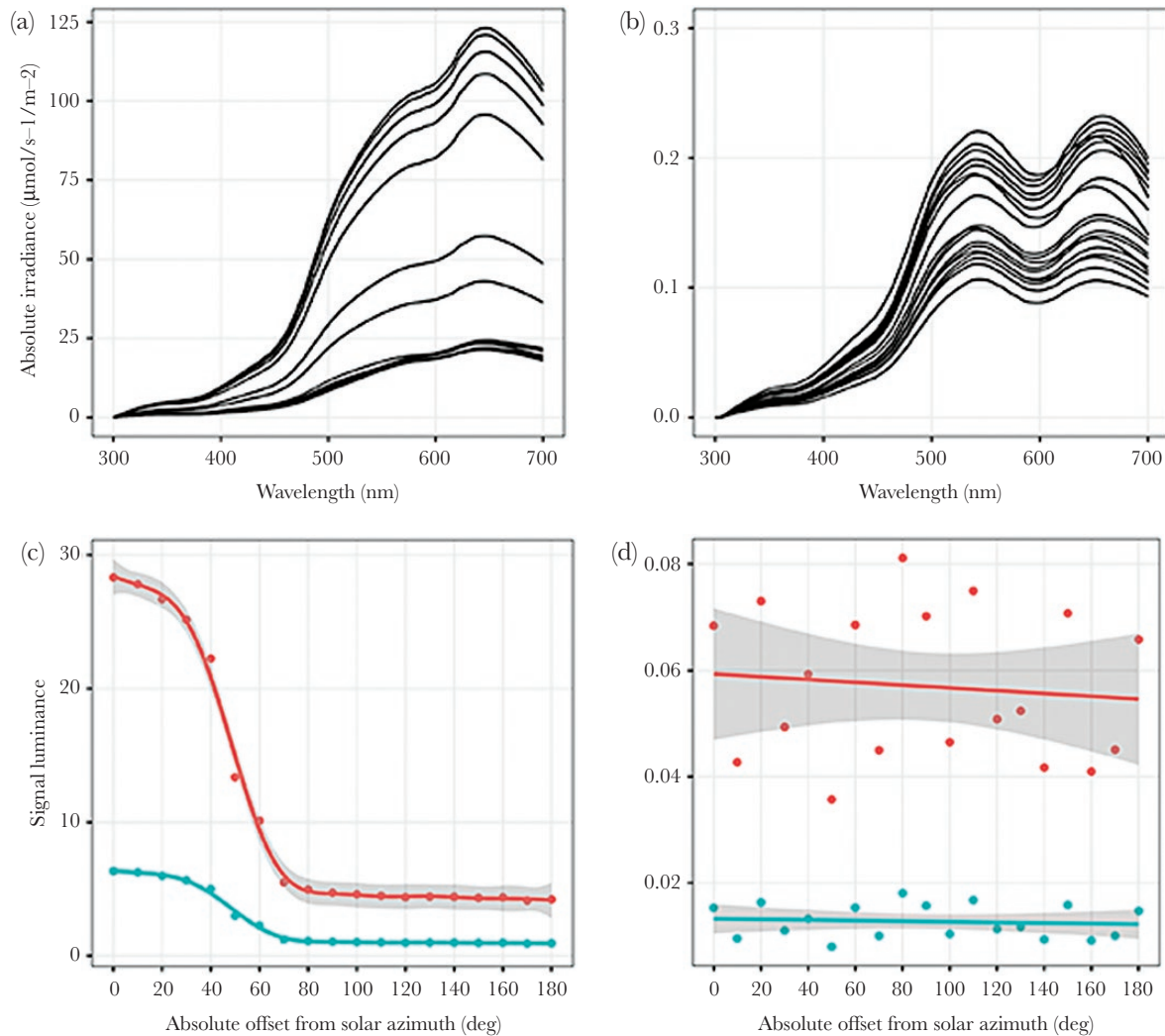


Figure 5

Solar irradiance and male signal luminance as a function of solar orientation. Solar irradiance in conditions of (a) full sun and (b) heavy cloud decreases with increasing offset from the sun, as recorded at 0° – 180° offset from the solar azimuth in 10° increments. Below is the luminance of male faces (red) and wings (green) across the same angular range, as modeled under full sun (c) or cloud (d). Lines denote generalized additive model fits \pm standard errors. Note differing y axes across all panels.

Because males' wings are also semitransparent, their optimal appearance is equally dependent on the background against which they are presented. Dark backgrounds—such as seaweed common to the foreshore habitats of *L. cana*—are fit for purpose, as they minimize transmitted light which would overwhelm the weakly reflective interference patterns. Recent work has documented just such an effect with males preferentially displaying against darker visual backgrounds, and the magnitude of signal/background contrast predicting mating success (White et al. 2020). Though we did not track mating success here a comparable effect on fitness is plausible, given that imprecision in either background selection or orientation relative to the sun will produce a similarly diminished signal (Figure 5c). It remains to be seen whether and to what extent the sun's position and visual backgrounds are weighed and integrated during courtship, but the accumulating evidence paints a striking picture of behavioral flexibility in service of communication efficacy.

The behaviors dedicated to the presentation of iridescent faces and wings imply an important role for the latter in sexual

communication, though their precise function is as-yet unresolved. As structurally colored ornaments the macro-scale appearance of faces and WIPs is closely tied to the nanoscale organization of underlying structures (Kinoshita 2008). If males differentially vary in their ability to secure the conditions and/or material required to generate such highly organized tissues, then the expression of facial and wing signals may be condition dependent (Zahavi 1975; Keyser and Hill 1999; Shawkey et al. 2003).

Among holometabolous insects, the nanostructures that generate structural coloration are built and fixed during ontogeny using the pool of resources gathered during the larval stage (Ghiradella 2009). Their final appearance may therefore reflect individual variation in foraging ability and condition more generally (e.g., Kemp and Rutowski 2007; Kemp 2008). In *L. cana*, the male's UV-white are a product of incoherent scattering by modified bristles (unpublished data, see Frantsevich and Gorb 2006 for details in a sister species), the brightness of which will largely depend on the size and density of scatterers (Giraldo and Stavenga 2007; White et al. 2012; Stuart-Fox

et al. 2018). The appearance of their WIPs is instead a product of coherent scattering, with color differences across the wing surface chiefly determined by variation in the thickness of the cuticular thin films (Shevtsova et al. 2011). Recent meta-analytic evidence supports the potential for heightened condition-dependent expression among structural colors in general (White 2020), and also suggests that the greater organization demanded by coherent, as opposed to incoherent, scatterers make them particularly suitable as condition-dependent ornaments. This argues for WIPs as more probable vectors of information on mate quality in *L. cana* than faces, with the latter potentially serving as signals of sex or species identity. Despite convincing evidence for sexual selection in WIP evolution (Katayama et al. 2014; Hawkes et al. 2019), direct tests of their function as signals remain outstanding. Simple binary or sequential mate-choice assays examining female preferences for manipulated males (perhaps via artificial thickening of the wings to modify and/or destroy their WIPs) would be valuable, as would rearing experiments testing the influence of juvenile development environments and resource limitation on the expression of WIPs and facial signals.

In addition to iridescent ornaments as conduits of information, a nonexclusive possibility is that male behavior is itself informative of mate quality. Courtship displays are likely to be energetically costly as the cycle of pursuing, straddling, and displaying with a given female is often repeated several times before a clear outcome is achieved. Males clearly vary in their ability to achieve and sustain optimal orientations during courtship (Figure 3). If this variation is dependent on male condition or “quality,” then their colored ornaments may simply serve as amplifiers of their behavioral performance. Displays against suboptimal backgrounds or at imprecise solar orientations would be readily apparent, particularly in the appearance of WIPs (Shevtsova et al. 2011). Potential benefits to selective females are predictable since, as predators, flight ability is central to prey capture in *L. cana* (Steidle et al. 1995; Pont 2019). This form of condition-dependent variation in courtship effort, and corollary benefits to choosy viewers, is well documented (Jennions 1998; Wagner and Hoback 1999; Kotiaho 2000). Comparable effects are known for variation in the precision of displays such as in the quality of courts constructed by bowerbirds (Endler et al. 2010; Kelley and Endler 2012), and the combination of limited-view iridescence and precision displays in *L. cana* creates the potential for a similar dynamic.

Our results extend a growing body of evidence, which suggests extensive coevolution between signals and display behaviors (Endler 1991; Endler and Thery 1996; Dakin and Montgomerie 2009; Bortolotti et al. 2011; Sicsu et al. 2013; Klomp et al. 2017; White 2017; Simpson and McGraw 2018). Notably, the strength of this link appears to vary with the extent of dynamism in signaling environments and/or signals. That is, signals and environments which vary across fine temporal or spatial scales are frequently coupled with flexible behaviors to enhance the efficacy of information exchange. Iridescent signaling systems—such as that examined here—fall at one extreme, with examples from insects (White et al. 2015), birds (Endler and Thery 1996; Dakin and Montgomerie 2009; Simpson and McGraw 2018), and guppies (Endler 1991), revealing considerable plasticity in display behavior for optimizing signal delivery. Environments which vary unpredictably across fine spatial and temporal scales appear to favor similar adaptive solutions. The foreshores of *L. cana* offer a case in point because a single wave can add or remove tracts of seaweed which the flies use for shelter and signaling, and thus entirely restructure their immediate visual environment.

Despite these few well-defined examples, the extent to which we can predict any one of behavior, habitat structure, or signal

design from knowledge of the others is a long-standing question (Poulton 1890; Lythgoe 1988; Endler and Thery 1996). One key to resolving this challenge is a deeper appreciation of the spatio-temporal complexity of visual communication. Exciting advances continue to be made in defining and integrating the spectral (van den Berg et al. 2020; Maia et al. 2019), spatial (Caves et al. 2018; Stoddard and Osorio 2019), and temporal (Gruson, Andraud, et al. 2019) features of signal production and perception, and systems such as *L. cana* offer a promising context for empirical progress.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data availability: Analyses reported in this article can be reproduced using the data and code provided by White and Latty (2020).

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REFERENCES

- van den Berg CP, Troschianko J, Endler JA, Marshall NJ, Cheney KL. 2020. Quantitative Colour Pattern Analysis (QCPA): a comprehensive framework for the analysis of colour patterns in nature. *Methods Ecol Evol.* 11:316–332.
- Bortolotti GR, Stoffel MJ, Galvan I. 2011. Wintering Snowy Owls *Bubo scandiacus* integrate plumage colour, behaviour and their environment to maximize efficacy of visual displays. *Ibis.* 153:134–142.
- Butterworth NJ, Byrne PG, Wallman JF. 2019. The blow fly waltz: field and laboratory observations of novel and complex dipteran courtship behavior. *J Insect Behav.* 32:109–119.
- Butterworth NJ, White TE, Byrne PG, Wallman JF. 2020. Love at first flight: wing interference patterns are species-specific and sexually dimorphic in blowflies (Diptera: Calliphoridae). *bioRxiv.* doi:10.1101/2020.02.18.948646
- Caves EM, Brandley NC, Johnsen S. 2018. Visual acuity and the evolution of signals. *Trends Ecol Evol.* 33:358–372.
- Coemans MA, Vos Hzn JJ, Nuboer JF. 1994. The relation between celestial colour gradients and the position of the sun, with regard to the sun compass. *Vision Res.* 34:1461–1470.
- Dakin R, Montgomerie R. 2009. Peacocks orient their courtship displays towards the sun. *Behav Ecol Sociobiol.* 63:825–834.
- Endler JA. 1991. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Res.* 31:587–608.
- Endler JA. 1992. Signals, signal conditions, and the direction of evolution. *Am Nat.* 139:S125–S153.
- Endler JA. 1993. Some general comments on the evolution and design of animal communication systems. *Philos Trans R Soc Lond B Biol Sci.* 340:215–225.
- Endler JA, Endler LC, Doerr NR. 2010. Great bowerbirds create theaters with forced perspective when seen by their audience. *Curr Biol.* 20:1679–1684.
- Endler JA, Thery M. 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. *Am Nat.* 148:421–452.
- Fleishman IJ, Leal M, Sheehan J. 2006. Illumination geometry, detector position and the objective determination of animal signal colours in natural light. *Anim Behav.* 71(2):463–474.

- Fleishman IJ, Ogas B, Steinberg D, Leal M. 2016. Why do *Anolis* dewlaps glow? An analysis of a translucent visual signal. *Funct Ecol*. 30(3):345–355.
- Frantsevich L, Gorb S. 2006. Courtship dances in the flies of the genus *Lispe* (Diptera: Muscidae): from the fly's viewpoint. *Arch Insect Biochem Physiol*. 62:26–42.
- Ghiradella HT, Butler MW. 2009. Many variations on a few themes: a broader look at development of iridescent scales (and feathers). *J R Soc Interface*. 6(Suppl 2):S243–S251.
- Giraldo MA, Stavenga DG. 2007. Sexual dichroism and pigment localization in the wing scales of *Pieris rapae* butterflies. *Proc Biol Sci*. 274:97–102.
- Gruson H, Andraud C, Daney de Marcillac W, Berthier S, Elias M, Gomez D. 2019. Quantitative characterization of iridescent colours in biological studies: a novel method using optical theory. *Interface Focus*. 9:20180049.
- Gruson H, White T, Maia R. 2019. Light: import spectral data and metadata in R. *J Open Source Softw*. 43:1857.
- Hardie RC. 1986. The photoreceptor array of the dipteran retina. *Trends Neurosci*. 9:419–423.
- Hawkes MF, Duffy E, Joag R, Skeats A, Radwan J, Wedell N, Sharma MD, Hosken DJ, Trosianko J. 2019. Sexual selection drives the evolution of male wing interference patterns. *Proc Biol Sci*. 286:20182850.
- Heinze S, Reppert SM. 2011. Sun compass integration of skylight cues in migratory monarch butterflies. *Neuron*. 69:345–358.
- Jennions M. 1998. Variation in courtship rate in the fiddler crab *Uca annulipes*: is it related to male attractiveness? *Behav Ecol*. 9:605–611.
- Johnsen S. 2012. The optics of life: a biologist's guide to light in nature. Princeton, NJ: Princeton University Press.
- Katayama N, Abbott JK, Kjærandsen J, Takahashi Y, Svensson EI. 2014. Sexual selection on wing interference patterns in *Drosophila melanogaster*. *Proc Natl Acad Sci USA*. 111:15144–15148.
- Kelly CD. 2006. Replicating empirical research in behavioral ecology: how and why it should be done but rarely ever is. *Q Rev Biol*. 81:221–236.
- Kelley LA, Endler JA. 2012. Illusions promote mating success in great bowerbirds. *Science*. 335:335–338.
- Kemp DJ. 2007. Female butterflies prefer males bearing bright iridescent ornamentation. *Proc Biol Sci*. 274:1043–1047.
- Kemp DJ. 2008. Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. *Evolution*. 62:2346–2358.
- Kemp DJ, Rutowski RL. 2007. Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. *Evolution*. 61:168–183.
- Keyser AJ, Hill GE. 1999. Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. *Proc R Soc B*. 266:771–777.
- Kinoshita S. 2008. Structural colors in the realm of nature. Singapore: World Scientific Publishing Company.
- Klomp DA, Stuart-Fox D, Das I, Ord TJ. 2017. Gliding lizards use the position of the sun to enhance social display. *Biol Lett*. 13:20160979.
- Kotiaho JS. 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behav Ecol Sociobiol*. 48:188–194.
- Land MF. 1993. The visual control of courtship behaviour in the fly *Poecilobothrus nobilitatus*. *J Comp Physiol A*. 173:595–603.
- Lebhardt F, Ronacher B. 2015. Transfer of directional information between the polarization compass and the sun compass in desert ants. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 201:599–608.
- Lunau K, Middelman A, Pianka M. 2006. Density- and food-resource-dependent courtship behaviour in the fly *Poecilobothrus nobilitatus* L. (Diptera, Dolichopodidae). *Entomologie heute*. 18:123–132.
- Lythgoe JN. 1988. Light and vision in the aquatic environment. In: Atema J, Fay RR, Popper AN, Tavolga WN, editors. Sensory biology of aquatic animals. New York, NY: Springer. pp. 57–82.
- Maia R, Gruson H, Endler JA, White TE. 2019. Pavo 2: new tools for the spectral and spatial analysis of colour in R. *Methods Ecol Evol*. 10:1097–1107.
- Marshall SA. 2012. Flies: the natural history & diversity of Diptera. Richmond Hill, Ontario: Firefly Books.
- Mouchet SR, Vukusic P. 2018. Structural colours in lepidopteran scales. In: Advances in insect physiology. Academic Press. Vol. 54. p. 1–53.
- Müller M, Wehner R. 2007. Wind and sky as compass cues in desert ant navigation. *Naturwissenschaften*. 94:589–594.
- O'Neill SJ, White TE, Lynch KE, Kemp DJ. 2019. Male guppies differ in daily frequency but not diel pattern of display under daily light changes. *Behav Ecol Sociobiol*. 73:157.
- von Philipsborn A, Labhart T. 1990. A behavioural study of polarization vision in the fly, *Musca domestica*. *J Comp Physiol A*. 167:737–743.
- Pont AC. 2019. Studies on the Australian Muscidae (Diptera). VIII. The genus *Lispe* Latreille, 1797. *Zootaxa*. 4557:1–232.
- Poulton EB. 1890. The colours of animals. London (UK): Paul, Trench, Trübner & Co.
- Prum R. 2006. Anatomy, physics, and evolution of structural colors. In: Hill GE, McGraw KJ, editors. Bird coloration. vol. 1: mechanisms and measurements. Cambridge (MA): Harvard University Press. pp. 295–353.
- R Core Team. 2018. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.
- Reppert SM, Guerra PA, Merlin C. 2016. Neurobiology of monarch butterfly migration. *Annu Rev Entomol*. 61:25–39.
- Shawkey MD, Estes AM, Siefferman LM, Hill GE. 2003. Nanostructure predicts intraspecific variation in ultraviolet-blue plumage colour. *Proc Biol Sci*. 270:1455–1460.
- Shevtsova E, Hansson C, Janzen DH, Kjærandsen J. 2011. Stable structural color patterns displayed on transparent insect wings. *Proc Natl Acad Sci USA*. 108:668–673.
- Sicsu P, Manica LT, Maia R, Macedo RH. 2013. Here comes the sun: multimodal displays are associated with sunlight incidence. *Behav Ecol Sociobiol*. 67:1633–1642.
- Simpson RK, McGraw KJ. 2018. It's not just what you have, but how you use it: solar-positional and behavioural effects on hummingbird colour appearance during courtship. (G. Grether, ed.). *Ecol Lett*. 21:1413–1422.
- Steidle JL, Dettner K, Hübner G, Aöpf K, Reinhard J. 1995. The predaceous fly *Lispe candicans* (Diptera: Muscidae) and its chemically protected prey, therove beetle *Bledius furcatus* (Coleoptera: Staphylinidae). *Entomol Gen*. 20:011–011.
- Stoddard MC, Osorio D. 2019. Animal coloration patterns: linking spatial vision to quantitative analysis. *Am Nat*. 193:164–186.
- Stuart-Fox D, Newton E, Mulder RA, D'Alba L, Shawkey MD, Igc B. 2018. The microstructure of white feathers predicts their visible and near-infrared reflectance properties. *PLoS One*. 13:e0199129.
- Thieurmel B, Elmarhraoui A. 2019. Suncalc: compute sun position, sunlight phases, moon position and lunar phase. R Package Version, 50.
- Vukusic P. 2006. Structural colour in lepidoptera. *Curr Biol*. 16:R621–3.
- Wagner WE, Hoback WW. 1999. Nutritional effects on male calling behaviour in the variable field cricket. *Anim Behav*. 57:89–95.
- Warrant EJ. 2016. Matched filtering and the ecology of vision in insects. In: Von der Emde G, Warrant E, editors. The ecology of animal senses. Cham, Switzerland: Springer. pp. 143–167.
- Weaver RJ, Santos ESA, Tucker AM, Wilson AE, Hill GE. 2018. Carotenoid metabolism strengthens the link between feather coloration and individual quality. *Nat Commun*. 9:73.
- Weir PT, Dickinson MH. 2012. Flying *Drosophila* orient to sky polarization. *Curr Biol*. 22:21–27.
- White TE. 2017. Jewelled spiders manipulate colour-lure geometry to deceive prey. *Biol Lett*. 13:20170027.
- White TE. 2020. Structural colours reflect individual quality: a meta-analysis. *Biol Lett*. 16:20200001.
- White TE, Latty T. 2020. Flies improve the salience of iridescent sexual signals by orienting toward the sun. *Behav Ecol*. doi:10.5281/zenodo.3985000.
- White TE, Macedonia J, Birch D, Dawes J, Kemp DJ. 2012. The nanoanatomical basis of sexual dimorphism in iridescent butterfly coloration. *Aust J Zool*. 60:101–107.
- White TE, Vogel-Ghibely N, Butterworth NJ. 2020. Flies Exploit Predictable Perspectives and Backgrounds to Enhance Iridescent Signal Salience and Mating Success. *Am Nat*. 195:733–742.
- White TE, Zeil J, Kemp DJ. 2015. Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. *Evolution*. 69:14–25.
- Zahavi A. 1975. Mate selection—a selection for a handicap. *J Theor Biol*. 53:205–214.
- Zeil J. 1983. Sexual dimorphism in the visual system of flies: the compound eyes and neural superposition in Bibionidae (Diptera). *J Comp Physiol*. 150:379–393.
- Zimmer M, Diestelhorst O, Lunau K. 2003. Courtship in long-legged flies (Diptera: Dolichopodidae): function and evolution of signals. *Behav Ecol*. 14:526–530.