

# A novel framework to study colour signalling to multiple species

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# Summary

1. The overwhelming focus of studies on communication has been on interactions among conspecifics. However, communication is often selected by a complex network of disparate intended perceivers and eavesdroppers belonging to multiple species. Shifting towards a multiple-perceiver paradigm requires a framework to compare the perception of signals across the different community members. Here, we present the *stimulation landscape*, a generalist model that achieves this goal.

**2.** A stimulation landscape consists of a multidimensional space describing every possible stimulus for a given signalling system, to which is added one dimension indicating for each stimulus its conspicuousness for a given perceiver. Random sampling of stimuli in the landscape then allows computing a reference distribution of conspicuousness, which is used to standardize the observed conspicuousness.

**3.** The stimulation landscapes corresponding to the different perceivers participating in a communication system all have the same dimensionality and scaling. They can thus be combined to describe the amalgamation of the selective forces exerted on a signal by the distinct sensory systems of multiple perceivers.

**4.** We detail the model in the context of colour signalling and apply it to the case of sexual communication in songbirds. Songbirds have a different visual system than their main predators, the birds of prey. We therefore asked whether songbirds evolved colour signals that can mediate the trade-off between sexual selection exerted by conspecifics and natural selection exerted by birds of prey. We show that yellow – not ultraviolet colours as previously thought – maximize the difference of conspicuousness to songbirds and to birds of prey, but that the perceptual similarities between these two groups generally hamper the evolution of private visual communication in songbirds.

**5.** The stimulation landscape is a valuable tool to investigate the role of communication in structuring the large networks of interactions between species. We further show that the stimulation landscape is related to the evolutionary model of adaptive landscape. By explicitly addressing communication in a community context, the stimulation landscape contributes to bridge the current gap between evolution and ecology.

**Key-words:** adaptive landscape, bird, colour space, colour vision, community, private communication channel, signal

# Introduction

Communication is ubiquitous, occurring in all organisms from unicellular to humans and in all types of interactions, from mutualisms and symbiosis to predation, parasitism and mate choice (Searcy & Nowicki 2005). In all these

a binary game between signallers and perceivers. Although this binary perspective has allowed elucidating fundamental aspects of communication, like the evolutionary stability of honesty and the co-evolutionary dynamics between signallers and perceivers (Maynard Smith & Harper 2003), it does not account for the multiple selective forces shaping communication systems that are most frequently

interactions, communication has been typically studied as

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embedded in a myriad of interactions (McGregor 2005). In this paper, we present a framework that allows comparing the perception of signals among perceivers and, more generally, studying quantitatively communication at the community level.

Colour signalling has been arguably the most studied channel of communication in ecology and evolution, producing insights on niche partitioning, community structuring and speciation (Hill & McGraw 2006; Bennett & Thery 2007). A major breakthrough in studies on colour signalling happened with the use of spectrometry. Spectrometers allow objective and quantitative measurement of colours. In particular, spectrometers can characterize ultraviolet radiations, which are now known to play significant roles in the communication among various organisms but which remain invisible to our eyes (Jacobs 1992). In the 1990s, researchers in ecology started applying the Principal Component Analysis (PCA) to natural reflectance spectra with the goal to summarize the large number of variables provided by spectrometers (e.g. Cuthill et al. 1999). The method was not new as it has been used in photographicscience application since the mid-twentieth century (Tzeng & Berns 2005). Today, PCA is still a widely applied method in colour technology to describe the physical property of colours (Tzeng & Berns 2005).

During the last decade, several articles cautioned about using PCA in studies in ecology and evolution because principal components (PCs) have no relationship with known visual mechanisms related to the sensation of colour perception (e.g. see Endler & Mielke 2005). An increasing number of studies thus used models of visual perception describing how we humans perceive colour stimuli. However, the perception of colours is dependent on the sensorial apparatus of animals, which varies among species (Ödeen, Håstad & Alström 2011). A second turning point in research on colour signalling thus arose with the use of species-specific models of colour perception. Such models contributed to significant advances in the field of animal communication. In particular, they have helped recognizing the major role of the sensory ecology of the perceiver in shaping the design of signals (Endler et al. 2005; Stoddard & Prum 2008). Such an advance contributed to embed ecologically orientated topics, like flower and fruit dispersal syndromes, into evolutionary frameworks, like the model of sensory bias (Lomáscolo et al. 2010).

A strong limitation to current models of colour perception is the impossibility to compare them across species. With these models, for example, one can study whether flower species A is more contrasting against the background (referred as 'conspicuousness' hereafter) to a bee pollinator than flower species B is, but one cannot ask whether species A is more conspicuous to a bee than it is to a butterfly. The main reason for this limitation is that different species have colour spaces with different dimensionalities. A colour space is a graphical representation of the perception of coloured stimuli (Chittka & Brockmann 2005). In a colour space, the distance between two colour loci is proportional to the conspicuousness of one colour seen against the other one. The dimensionality of a colour space is mainly determined by the number of photoreceptor types used to perceive colours. For examples, while most birds and flies are tetrachromats (i.e. they use four types of photoreceptors), Old World primates and bees are trichromats and most mammals and ants are dichromats (Osorio & Vorobyev 2008). Inconveniently, distances in a four-dimensional colour space usually do not span equally as in a two- or threedimensional space. Furthermore, there is no psychophysical basis to assume that the maximal distance in a four-dimensional colour space corresponds to a pair of colours that are perceptually more conspicuous against each other than the pair with the maximal distance in a lower-dimensional space. On the contrary, a number of studies have revealed that at the individual level, capacities in perceiving colour distances are regulated by previous experiences with colour stimuli (e.g. in bumblebees; Dyer & Chittka 2004), thereby highlighting the need to consider perceptual distances between colours as a relative notion.

The impossibility to compare perceptual spaces strongly hampers their application in ecology and evolution because communication systems usually involve multiple species. This is most obvious in the generalized interactions that involve dozens of species and that characterize most mutualisms (Bascompte & Jordano 2007). But even in more specialized interactions, such as when one individual intentionally interacts with individuals of the same species as in male-female sexual interactions, communication is influenced by other species such as predators and parasites (Endler 1980; Hamilton & Zuk 1982). Indeed, core paradigms on communication among the sexes such as the handicap principle and the Hamilton-Zuk hypothesis explicitly assumes that the effects of predators and parasites render epigamic signals informative to potential mates (Hamilton & Zuk 1982). Consequently, signals result from multiple and possibly conflicting selective forces, which can be exerted by distinct species (Endler 1980).

In this article, we introduce the *stimulation landscape*, a new model combining the benefits of PCA and models of colour perception, which allows comparing the conspicuousness of signals across species. The first section presents the model. As an example of application, we then use the stimulation landscape to compare the perception of colours between songbirds and birds of prey. Specifically, we re-evaluate the suggestion that ultraviolet signals could function as a private communication channel in songbirds (Guilford & Harvey 1998; Håstad, Victorsson & Ödeen 2005). In the discussion, we will argue that the stimulation landscape is a powerful framework that can be applied to study many communication systems with multiple perceiving species, including those using non-visual senses.

#### The model of stimulation landscape

#### MODEL OVERVIEW

The goal of the model is to make interspecific comparisons of the perception of a given coloured signal. The perception of coloured signals is modelled using a colour space. We assume that colour spaces vary between but not within species. This is not strictly true given that studies have evidenced substantial interindividual variation in the perception of colours (Chittka & Brockmann 2005). However, it is still unknown how important this intraspecific variation is. We further assume that most of the variation between colour spaces is determined by the number of photoreceptor types used to process colour stimuli, which describes the dimensionality of the colour space (Osorio & Vorobyev 2008). To compare colour spaces among species, it is thus necessary to work with spaces whose dimensionality is identical for every species participating in a communication process.

Colour stimuli are characterized by their reflectance spectra. In a first step, we represent colour stimuli in a spectral space, which represents the possible physical variation in colour stimuli (Fig. 1 step 1a). Spectral spaces are interesting in that they have a fixed, predefined dimensionality (e.g. 401 dimensions if the percentage of reflected light is given for each nanometre within the range 300– 700 nm); however, they are uninformative about how species perceive stimuli. The next step is then to combine information on the perception of stimuli with a spectral



Fig. 1. Construction and use of the stimulation landscape.

space. Here, one is interested by the conspicuousness of a visual stimulus when perceived against a background stimulus. In a colour space, setting the background stimulus to constant allows describing the conspicuousness of the signalling stimulus with a unique variable (Fig. 1 step 1b). A stimulation landscape then consists of a spectral space plus one dimension that indicates, for each stimulus, its conspicuousness when seen against another fixed stimulus (Fig. 1 step 1c). Within a given communication system, the dimensionality of the stimulation landscape is identical among perceivers. 'Peaks' in the landscape indicate colour stimuli that are maximally conspicuous, while 'valleys' indicate inconspicuous stimuli similar to the background coloration.

Estimates of conspicuousness are eventually standardized within the interval [0, 1]. This is because in most colour spaces, the dimensionality of the colour space determines the values of conspicuousness. Put differently, with the widely used formulae provided by Kelber. Vorobyev & Osorio (2003) to model colour spaces, the distance from the centre of a triangle (for trichromatic vision) to one of its apices is 0.82, while the distance from the centre of a tetrahedron (for tetrachromatic vision) to one of the vertices is 0.87. Standardization of conspicuousness is achieved by randomly sampling a large number of stimuli (usually  $>10^3$ ) in the stimulation landscape and by substituting the original colour distances between the stimuli and the background by their cumulative density, through the computation of the empirical cumulative distribution of the original distances (Fig. 1 step 2). Indeed, if the distribution of conspicuousness across the stimulation landscape was uniform or Gaussian, we could standardize it based on maximum and minimum, or on z-transformation, respectively. Because there is no a priori reason to expect such simple distributions, we standardize conspicuousness using the more general method, that is, the quantile functions based on the cumulative distributions. Estimate of conspicuousness into such standardized value corresponds to the fraction of randomly sampled values that are lower or equal to a given value of conspicuousness. For example, if a colour with an original conspicuousness of 2.5 has a standardized value of 0.3, it implies that 30% of the simulated colours have conspicuousness lower or equal to 2.5.

The constant dimensionality of stimulation landscapes among perceivers allows broad applications that go beyond the mere comparison of visual abilities between species. For example, stimulation landscapes from perceivers with different sensory systems can be added, subtracted or weighted to create synthetic landscapes that describe the amalgamation of the combined or relative stimulation of multiple perceivers participating in communication systems (Fig. 1 step 3).

#### DIMENSIONALITY OF THE SPECTRAL SPACE

Although the dimensionality of a spectral space is typically high, it can be reduced considerably. For two reasons, it is helpful to reduce the dimensionality of a spectral space to build a stimulation landscape. First, on practical grounds, data exploration is strongly facilitated in low-dimensional parameter spaces. Exploring the shape of the landscape, for example to identifying global and local 'peaks', will require to optimize a function linking the standardized conspicuousness to the variables describing the spectral space. Speed and accuracy of optimization algorithms are strongly enhanced if the number of free parameters in the function to optimize is reduced. Second, a low-dimensional spectral space is necessary to account for the constraints that underlie the physical process of light reflection and thus to standardize the conspicuousness of signals using biologically realistic simulated colours (see Discussion and Fig. S1 in Supporting information). Indeed, standardization requires simulating reflectance spectra by sampling random values at each variable that characterizes the spectral space. As reflectance spectra typically consist of a large number of values, a spectral space could easily be described with hundreds of variables. However, randomly sampling hundreds of variables independently would generate spectra with numerous sharp peaks (Fig. S1a, Supporting information), which do not resemble the highly autocorrelated and thus smoothly shaped spectra observed in nature (Fig. S1d, Supporting information; Jaaskelainen, Parkkinen & Toyooka 1990).

We propose using the PCA to recover the 'ecologically relevant dimensionality' of the spectral space (ERD hereafter), that is, the minimal number of variables in a spectral space that is needed to characterize all the perceptible variation among the reflectance spectra. Recovering the ERD follows a three-step iterative process. First, a PCA is applied to the spectral data set to calculate PC scores and to define a linear combination of principal components. Second, a complete spectral data set is reconstructed using the scores and the linear combination of *i* principal components. Third, the fit between original and reconstructed data sets is calculated to estimate the accuracy of data reconstruction. These three steps are repeated increasing *i* (Figs. S2 and S3, Supporting information).

In more details, treating a data set of q reflectance spectra as a matrix S of size  $k \ge q$ , with k corresponding to wavelength, principal components are linear combinations of the eigenvectors  $e_i$  with nonzero eigenvalue ( $i \in [1,h]$ ;  $h \leq k$ ) of the variance-covariance matrix of S. Coefficients of the linear combinations are such that PC1 has the largest possible variance, and each succeeding principal component has the highest variance while being constrained to be orthogonal to the preceding component. A reflectance spectrum of S can be exactly described by the linear combination of PCA scores including all of the hcomponents because this combination describes the complete spectral space after rotation. If the number of principal components is lower than h, a reflectance spectrum of S can still be reconstructed but with a certain degree of imprecision depending on the number and rank of the principal components used in the linear combination. The

linear combination of PCA scores describes indeed a subspace of the spectral space (for details, see Tzeng & Berns 2005).

Applying this method and estimating statistically the fit between original and reconstructed data sets, studies in colour technology revealed that between five and eight principal components are enough to characterize variation among the reflectance spectra of natural surfaces over the range 400-700 nm (Maloney 1986; Jaaskelainen, Parkkinen & Toyooka 1990). The studies in ecology, the selected number of components depends on the identity of the perceiver. For example, it will be higher for species that can discriminate among thousands of colours than for species possessing achromatic black-and-white vision only. We thus propose to evaluate the fit between reconstructed and original spectra through the similarities between the two types of spectra as perceived by the species with the best abilities to discriminate colours among the perceivers participating in communication. The ability to discriminate between original and reconstructed spectra is implemented using a psychophysical model of colour discrimination. The ERD then corresponds to the minimal number of principal components necessary, so that a high ratio (set to 0.95 in this study) of reconstructed spectra is not perceived as different from original spectra by a given perceiver (Figs. S2 and S3, Supporting information).

Psychophysical models of colour discrimination are available for few animal species only. The terrestrial animal with the highest known colour discrimination abilities is the butterfly *Papilio xuthus* (Koshitaka *et al.* 2008). Using a large data set of thousands of floral reflectance spectra, we determined eight dimensions as the ERD of the spectral space of *P. xuthus* (data not shown). Eight dimensions should therefore be sufficient to accurately describe most spectral spaces. Given that eight components still allow simulating smooth-shaped reflectance spectra (the main reason to reduce the dimensionality of the spectral space), we recommend setting the ERD to eight if no model of colour discrimination is available.

#### Application to sexual signalling in birds

Two main types of visual systems are classically recognized among diurnal birds: the ultraviolet-sensitive and the violet-sensitive systems (Renoult, Courtiol & Kjellberg 2009; Ödeen, Håstad & Alström 2011; Aidala *et al.* 2012). The two systems differ in a number of parameters including the sensitivities of ultraviolet and blue photoreceptors, peaking at 367 and 444 nm, respectively, in the ultraviolet-sensitive system and at 412 and 452 nm in the violet-sensitive system (Hart & Vorobyev 2005). Among the species studied so far, most songbirds possess the ultraviolet-sensitive system, while birds of prey, the main predators of songbirds, possess the violet-sensitive system (Ödeen, Håstad & Alström 2011; Aidala *et al.* 2012). The differences in peak sensitivities between ultraviolet- and violet-sensitive systems have led several authors to conclude that ultraviolet signals could function as private channels of communication in songbirds, that is, they could have evolved to communicate with conspecifics while minimizing detection by avian predators (Guilford & Harvey 1998; Håstad, Victorsson & Ödeen 2005). In this section, we will use stimulation landscapes to compare coloration of epigamic signals as seen through the ultraviolet- and violet-sensitive visual systems and to re-examine the private channel hypothesis. We chose this case of private communication because it is a textbook example that has nevertheless remained contentious (Göth & Evans 2004; Stevens & Cuthill 2007).

### Materials and Methods

We studied the coloration of males and females of 51 bird species (38 with ultraviolet-sensitive and 13 with violet-sensitive colour vision) selected from the review of Hill (2006; pp. 140-148, Table 4.1), who compiled studies on sexual selection in birds involving visual communication. Each species was assigned either to the ultraviolet-sensitive or violet-sensitive visual system based on their position in avian phylogenies (Hackett et al. 2008) and on data on the visual system obtained with microspectrophotometric or molecular analyses in related species (Ödeen, Håstad & Alström 2011; Aidala et al. 2012). For each species, we measured coloration of the same five body parts in 3–5 males (mean =  $4.8 \pm 0.48$ ) and 1–5 females (mean =  $4.2 \pm 1.12$ ): breast, wings (greater coverts), ear coverts, back (mid-distance between rump and nape) and tail. In addition, we measured the colour of other body parts (e.g. wing stripes, necklace, forehead; see complete list in Table S1, Supporting information) that had been demonstrated to be sexually selected by females in studies listed in Hill's table (n = 99 studies). The overall data set consists of 568 feathered body parts including 89 epigamic (i.e. sexually selected) traits (Table S1, Supporting information). Spectral measurements were performed on museum skins, with a similar method as described in Schaefer, Schaefer & Vorobyev (2007).

PCA was run with the 568 reflectance spectra to reduce the dimensionality of the spectral space. To identify the ERD of the spectral space, perceptual similarities between original and reconstructed spectra were evaluated using the receptor noise limited (RNL) model of colour discrimination (Vorobyev & Osorio 1998). The RNL model is a psychophysical model of colour vision assuming that detection is mediated by chromatic mechanisms and that detection thresholds are set by noise originating in photoreceptors used in colour vision. Noise-related parameters were similar to those used in Schaefer, Schaefer & Vorobyev (2007) and the detection threshold was set to 1 JND. We used two sets of photoreceptor capture functions, corresponding either to an average ultraviolet- or an average violet-sensitive visual system (for details, see Endler & Mielke 2005). The illuminant spectrum was a standard CIE D65 (CIE Pub 1986: Table 1-1).

Colour conspicuousness was evaluated using the Goldsmith's model of colour vision (Goldsmith 1990). The Goldsmith's (1990) model attempts to reconstruct a colour space of colour vision based on the reflectance spectra of stimuli and the photoreceptor capture functions (same functions as above). We defined conspicuousness as the distance between stimulus loci and the centre of the colour space, which is equivalent to setting the background as an achromatic stimulus. We repeated the analyses using another model of colour vision (Endler & Mielke 2005), which requires specifying an illuminant (CIE D65) and a background coloration (mean spectrum of leaf measurements). Given that both models yielded similar results, only those obtained with the simpler Goldsmith's (1990) model are presented hereafter (see the Discussion section and Stoddard & Prum 2008). We first used a Kolmogorov–Smirnov (KS) test to compare the range of values of colour conspicuousness between ultravioletand violet-sensitive systems and thus to evaluate whether standardization was necessary before comparing both visual systems. We calculated non-standardized distances between background and 10<sup>5</sup> colours that were randomly simulated within the spectral space of ultraviolet- and violet-sensitive visual systems. Simulations were performed by randomly sampling each principal component within the interval defined by extreme scores found with the original spectra. Because the conspicuousness of random colours were consistently different between both visual systems (see results), we standardized conspicuousness before comparing these systems with each other using the procedure described previously.

To illustrate how stimulation landscapes can be used, we first identified which colours show maximal conspicuousness in the standardized stimulation landscapes of ultraviolet- and violet-sensitive systems. Global and local maxima in the landscape were identified by optimization using the DEoptim function (Ardia & Mullen 2010) of the DEoptim package for R (R Development Core Team 2011). DEoptim allows the estimation of the scores at the principal components maximizing the standardized conspicuousness. Then, we studied whether colour signals evolved to be conspicuous to mates in both birds with ultraviolet-sensitive and violet-sensitive visual systems. Working with standardized colour distances, we compared the conspicuousness of epigamic and nonepigamic traits. To account for a potentially different level of phylogenetically induced non-independence among epigamic traits and among non-epigamic traits, the comparison was performed using Generalized Estimating Equations (GEE) as implemented in the R package ape (Paradis, Claude & Strimmer 2004); GEE are an extension of generalized linear models for (phylogenetically) correlated data. Conspicuousness was defined as the continuous dependent variable and the type of visual system as a categorical independent variable. We selected a poisson family with a log-link function, and a phylogenetic tree of the 568 body parts was used to calculate the correlation matrix (see Data S1, Supporting information). Last, we studied whether birds with ultraviolet-sensitive systems have evolved private channels of communication that simultaneously maximize conspicuousness to conspecifics and minimize it to avian predators. We built a function calculating the difference of standardized conspicuousness between ultraviolet- and violet-sensitive visual systems. This difference was treated as one variable added to the spectral space to build a synthetic stimulation landscape as in Fig. 1 step 3. We then used DEoptim to locate the global peak in this synthetic landscape, that is, to identify the colour that would best function as a private channel of communication in songbirds. Finally, we tested (KS test) whether epigamic traits sported by species with ultraviolet-sensitive vision have higher standardized colour distances for the ultraviolet-sensitive systems compared with the visual system of predators, which would be indicative of a private communication channel.

#### **Results and Discussion**

Using either the ultraviolet- or the violet-sensitive visual system, we found that five components allowed more than 95% of reconstructed spectra to be perceptually identical to the original spectra. As it typically occurs when using a PCA with reflectance spectra (Maloney 1986), the first principal component was highly correlated with brightness, which is the achromatic component of colour stimuli, whereas the remaining components described variation in spectral shape, that is, the chromatic component of colour stimuli (result not shown). Because we were only interested in the chromatic component of colour stimuli, we stan-

dardized reflectance spectra to have integrals of constant value before performing the PCA again. With standardized reflectance spectra, only four components allowed more than 95% of reconstructed spectra to be perceptually identical to the original spectra (Fig. S3, Supporting information). The ERD of the spectral space was thus set to four, and subsequent analyses were performed using standardized spectra.

Based on non-standardized colour distances, randomly simulated colours were less conspicuous for the violet-sensitive system than they were for the ultraviolet-sensitive system (KS test: D = 0.04, P < 0.001; Fig. 2a). The systematic bias of the violet-sensitive system towards lower values of perceived conspicuousness requires rescaling conspicuousness in both ultraviolet and violet-sensitive systems to render them comparable. In non-epigamic plumage colours, we found that the majority of standardized distances have very low values (42% and 80% of standardized distances are below 0.1 and 0.3, respectively; Fig. 2b). The distributions of standardized distances from colour loci to the centre of the colour space are significantly different between epigamic and non-epigamic traits ( $n_{epig} = 89$ ;  $n_{other} = 479$ ; GEE: effect = 0.58, F = 31.7, P < 0.001; Fig. 2c). Epigamic traits are more conspicuous, indicating that they have been adapted to stimulate the sensory system of mates. The difference between epigamic and non-epigamic distance distributions is likely due to the significantly higher proportion of standardized distances above 0.3 (Fig. 2d) in epigamic (51%) compared with non-epigamic (21%) colours (GEE with 'above/below 0.3' and 'epigamic/non-epigamic' as dependent and independent variables, respectively, and a binomial family with logit link function: effect = 1.57, F = 42.9, P < 0.001). Consequently, we will use 0.3 as a threshold to define adaptation to the sensory system of perceivers and thus conspicuousness.

Epigamic traits sported by species with ultraviolet-sensitive vision (n = 66) had marginally higher standardized distances for the ultraviolet-sensitive systems compared with the visual system of predators (KS test: D = 0.27, P = 0.09). However, this result vanished when we considered colours adapted to the sensory system of mates only (i.e. with standardized distances above 0.3; n = 41; D = 0.24, P = 0.17). Unlike a previous study (Håstad, Victorsson & Ödeen 2005), our results show that, on average, epigamic colour signals in ultraviolet-sensitive species have not evolved to exploit a private channel of visual communication. The strong similarity between ultraviolet- and violet-sensitive visual systems could explain this result given that the stimulation landscapes of these two systems have very similar global (red colour) and local (blue colour) peaks (Fig. 3a). Thus, any increase towards more conspicuousness to mates in songbirds would increase also the conspicuousness to predators. The strong overlap in the perception of colours between intended and unintended perceivers may thus be an important constraint to the possibility that the trade-off between sexual and



**Fig. 2.** Density distribution of simulated and measured estimates of colour conspicuousness of bird plumage. Panel (a) shows the density distribution of simulated  $(n = 10^5)$  colour distances between signals and the centre of the bird colour space calculated with an ultraviolet-(black line) and a violet-sensitive visual system (grey line). Panel (b) and (c) show the density distribution of measured standardized distances for non-epigamic traits (n = 479) and epigamic traits (n = 89), respectively. Panel (d) illustrates the difference between density distributions given in (b) and (c). Positive bars indicate colour distances that are relatively more frequent in epigamic traits than in non-epigamic traits.

natural selection is mediated by private communication channels in songbirds.

In a synthetic landscape that describes the difference in colour conspicuousness perceived by ultraviolet- and violetsensitive systems, the peak (yellow colour without ultraviolet reflection) is located in the lower right quadrant (Fig. 3b). Strikingly, the green, ultraviolet-green, yellow and ultraviolet-yellow colours that are in that quadrant are only exhibited by species having ultraviolet-sensitive vision. Those colours are therefore likely responsible for the following phenomenon: among the colours that are more conspicuous for ultraviolet-sensitive species than for violetsensitive species, there are significantly more epigamic traits (28.0%) in ultraviolet-sensitive species than in violet-sensitive species (10%; GEE with the type of visual system and 'epigamic/non-epigamic' as dependent and independent variables, respectively, and a binomial family with logit link function: effect = 0.6, F = 7.28, P = 0.016). This result suggests that colours rich in middle wavelengths could contribute to private communication in some songbirds and other bird species with ultraviolet-sensitive vision, although this suggestion awaits confirmation in future studies because 62% of the colours that were more conspicuous for ultraviolet-sensitive species were non-epigamic traits.

The finding that yellow without ultraviolet reflection but not ultraviolet colours maximize the difference of conspic-

uousness to songbirds and to birds of prey contradicts the classical thought that a private communication channel in songbirds would most likely involve ultraviolet reflecting signals (Guilford & Harvey 1998; Håstad, Victorsson & Ödeen 2005). We suggest a functional explanation for this result that involves two separate mechanisms. First, colours are in general highly conspicuous to tetrachromatic species if they strongly stimulate two receptors and minimize the stimulation of the other two receptors (Gomez & Thery 2007). In the blue peak, for example, only the two photoreceptors for short wavelengths are strongly stimulated. This explains why adding an ultraviolet peak to yellow decreases conspicuousness to the avian eye (Gomez & Thery 2007). Second, the second and third photoreceptors have a greater overlap in violet- compared with ultraviolet-sensitive vision. Owing to this differential overlap, yellow colours stimulate more strongly the third and fourth photoreceptors relative to the other two receptors in the ultraviolet- than in the violet-sensitive visual system. Thus, our results suggest that if some songbird species have evolved a communication channel that simultaneously maximizes colour conspicuousness to conspecifics while minimizing conspicuousness to predators, colour signals with high reflectance of the middle-range wavelengths are more likely to be produced than signals with high-ultraviolet reflectance.



**Fig. 3.** Distribution of epigamic colour signals of birds in the first two dimensions of the stimulation landscapes. The first two dimensions together explain 86.8% of the variation in colour spectra. Values along PC1 and PC2 are interpreted using the relations between wavelengths and wavelength coordinates in the PCA provided in Fig. S4 (Supporting information). Briefly, positive and negative PC1 values are associated with long (red) and short (blue) wavelengths, respectively, and positive and negative PC2 values are associated with long or short and middle (yellow and green) wavelengths, respectively. Epigamic signals of ultraviolet- and violet-sensitive species are indicated by black-and-white filled circles, respectively. In panel (a), black-and-white triangles show the location of the optima (large triangles: global; small triangles: local) calculated by optimizing PCA components in ultraviolet- and violet-sensitive visual systems, respectively. The coloration of the landscape indicates the conspicuousness at each locus within the first two dimensions of the stimulation landscape of an ultraviolet-sensitive system. In panel (b), the white triangle locates the global peak of the synthetic landscape calculated from the difference between standardized ultraviolet- and violet-sensitive conspicuousness. The coloration of the landscape indicates differential standardized conspicuousness, with positive values corresponding to colours that stimulate the ultraviolet-sensitive system more than the violet-sensitive system. The slight discrepancy between the locations of triangles and the shadowing for both panels is explained by the need to keep two dimensions of the spectral space constant for illustrative purpose (set to mean PC scores). White insets provide the reflectance spectra of the colours corresponding to the peaks (of ultraviolet-sensitive landscape only in panel (a); abscissa: wavelengths in nm; ordinate: reflectance).

#### GENERAL DISCUSSION

We presented a new model, the stimulation landscape, designed to perform quantitative studies of visual communication occurring at the level of communities. The model merges a spectral space that describes visual stimuli and a colour space where the information about conspicuousness of a coloured signal is summarized into a single dimension. In the following, we discuss the assumptions underlying model construction and their implications for studies in ecology and evolution.

#### MODEL ASSUMPTIONS

The stimulation landscape requires modelling a colour space to estimate the conspicuousness of a signal. In the example on bird coloration, we used the Goldsmith's (1990) model of colour vision that incorporates reflectance spectra and the photoreceptor capture functions only. Several eye parameters not accounted by this model are known to influence colour vision in birds and to vary between species (e.g. the relative density of photoreceptor types; Kelber, Vorobyev & Osorio 2003; Endler & Mielke 2005). In addition, other visual properties not modelled here like achromatic vision, temporal and spatial visual resolution and configuration of the visual field also impact the perception of coloured signals. Last, the Goldsmith's

model does not consider the chromatic adaptation of photoreceptors to the irradiance spectrum even though birds sometimes display under illuminants carefully selected (Endler & Thery 1996). The results presented here should be interpreted with these limitations in mind.

Although we used the Goldsmith's model with photoreceptor capture functions, which account for both photoreceptor sensitivities and the transmission of oil droplets, the Goldsmith's model can be used knowing photoreceptor sensitivities only (Kelber, Vorobyev & Osorio 2003). This has one obvious benefit: given the growing knowledge of photoreceptor sensitivities and of phylogenetic relationships allowing inferences of sensitivities in non studied species (Osorio & Vorobyev 2008), the Goldsmith's model and thus the model of stimulation landscape could both be applied to a large number of species. In addition, Stoddard & Prum (2008) compared the Goldsmith's (1990) model for birds with its extended version that additionally incorporates an ambient light spectrum, a mechanism of colour constancy induced by the background coloration and a log-transformation of the photoreceptor colour channels (Endler & Mielke 2005). These authors concluded that none of the additional parameters was necessary; arguing that selecting an appropriate model of colour space for comparative analyses requires a trade-off between pragmatic benefits, computational complexity and generality. Even though the model of stimulation landscape is a

flexible framework that could incorporate any model of colour vision in the future, we suggest that models of photoreceptor excitation space are valuable tools to estimate signal conspicuousness in most comparative analyses.

In some situations, for example when one is interested to know which colours are equally conspicuous to two visual systems (Fig. 1 step 3), it is appropriate to use a colour space with a known detection threshold. The detection threshold is then standardized like any other distance in the colour space. A limitation of the stimulation landscape is that the detection threshold cannot vary across the colour space. Yet, it is a current and conservative practice in studies in ecology to define a constant threshold value, which corresponds to the lowest threshold identified across the colour space (e.g. see Dyer *et al.* 2012).

A current issue may be that although stimulation landscapes can be compared among perceivers of a given communication system, they cannot be compared across independent analyses because PCA values vary according to the set of colours used to create spectral spaces. Obviously, one could always use the same standard data base of natural reflectance spectra to create standard spectral spaces. However, among the different data bases available online (listed in Kohonen, Parkkinen & Jaaskelainen 2006), none currently includes a high number of spectra measured in the range of 300-700 nm. In addition, variation of the spectral space built from a standard data base does not necessarily correspond to biologically meaningful variation for a given visual task. For example, pollinators have a tendency to visit a single plant species during one foraging bout (Waser 1986). Pollinators visiting a population of a blue flowering species would thus standardize conspicuousness within the blue variation only, which means that including red or yellow spectra within the reference set of spectra could be mostly irrelevant. Consequently, we suggest that constructing a stimulation landscape from a data set of spectra measured in each biological system is currently the best approach even though it prevents cross-study comparisons. The experimenter should define a priori which kinds of stimuli are relevant or not in a given visual task.

Crucially, the emitted signals simulated in the spectral space have to be biologically realistic to be used for standardization. This is ensured by simulating smoothly shaped spectra. Simulated spectra are smoothed because they are generated from a low number of parameters that are further bounded by real extreme values. A comparison of the distribution in the bird colour space of the 568 measured spectra with that of 568 spectra simulated from the spectral space illuminates important properties of these simulated spectra (Fig. 4). The cloud of simulated colours occupies a substantially larger volume in the colour space compared with the cloud of measured colours. This difference is mainly due to a higher diversity of hues in simulated colours, while the average chroma (i.e. saturation of colours) is moderately increased in simulated compared with measured colours. The lack of highly saturated col-



Fig. 4. Comparison of the perception of observed and simulated colour stimuli. The 568 spectra measured in birds (panel a) and 568 spectra simulated randomly in the spectral space (panel b) were projected into a tetrahedral colour space representing the colour perception of birds with an ultraviolet-sensitive system. Upper figures represent the volume occupied by the perceived colours within the colour space. For details on modelling the colour space and calculating volume, hue disparity and average chroma, see Stoddard & Prum (2008). The lower figures depict the Mollweide projection of colours from the tetrahedron to its encapsulating sphere, which allows visualizing the diversity of hues independently of chroma. u, s, m and l indicate to the ultraviolet-, short-, medium- and long-wavelength sensitive photoreceptors, respectively. Indices were calculated and figures were drawn using the R package pavo (Maia, Eliason & Bitton 2012).

ours in simulated spectra is important because these would indicate spectra with sharp peaks of reflectance. Indeed, with the exception of pure ultraviolet and pure red colours, natural colours are typically not highly saturated (Stoddard & Prum 2011). This explains why randomly sampling throughout the colour space to draw a reference set of spectra is not a suitable alternative to the method proposed here. In contrast, increasing the diversity of hues is necessary to generate reflectance spectra not included in the original data set but that are nevertheless biologically realistic. For example, although in living birds there is, to our knowledge, no known spectrum consisting of a first reflectance peak located around 350 nm and a second peak located around 510 nm, a colour corresponding to such a spectrum could be theoretically produced by adding a few turacoverdin pigments (peaking around 510 nm in Tauraco corythaix; Dyck 1992); over arrays of dermal collagen fibrils in bare skins (which maximally reflect around 350 nm in Tragopan temminckii; Prum & Torres 2003; but see Shawkey, Morehouse & Vukusic 2009; for non-additive interactions between colour-producing materials). This combination of spectral characteristics may be absent in the original data set because it is not achievable by natural systems (e.g. due to physiological, genetic or phylogenetic constraints) or because of evolutionary contingency (Stoddard & Prum 2011). However, it may also be absent because of incomplete sampling of signal variation in the communication system. Owing to this possibility, we think that these composite spectra should be included in the reference distribution of conspicuousness.

# FURTHER APPLICATIONS IN ECOLOGY AND EVOLUTION

We demonstrated how a quantitative comparison of the perceptual abilities of multiple perceivers allows testing the private communication hypothesis in a system with two antagonistic perceivers. The stimulation landscape can also be applied to study visual signalling with an entire community of perceivers. Understanding the mechanisms structuring the large networks of interactions between community members has become a challenge in ecology. Although communication has been assumed to be a major determinant of the structure of ecological interactions (e.g. Olesen et al. 2007), support for this conjecture is still lacking. By comparing the phenotype matching between signallers and perceivers across species, the stimulation landscape will allow quantifying the relative contributions of communication and other factors like neutral processes or the phylogenetic relatedness in the structure of interaction networks.

The model further offers a tool to quantitatively study the evolution of visual signals. As mentioned previously, numerous studies demonstrated a link between the fitness of the signaller and its conspicuousness, but there appears to be substantial variation in this relationship. The variation may be due to a number of parameters, including physiological (e.g. variation in the relationship between photoreceptor excitation and perceptual differences), psychological (e.g. variation in a threshold of conspicuousness above which different signals elicit similar responses) and ecological factors (variation between communication systems like between mimicry and aposematism and varying distance between signal and perceiver). The model of the stimulation landscape can be built from variously transformed distributions of conspicuousness, which allows modelling each of these parameters and evaluating their importance by comparing conspicuousness with empirical estimates of individual fitness. Interestingly, developing a stimulation landscape in this direction will approximate it to an adaptive landscape for phenotypic traits, in which the conspicuousness axis is replaced by an indicator of individual fitness (Simpson 1944). Contrary to most adaptive landscapes, however, the shape of the stimulation landscape is not estimated statistically using a regression model (Arnold, Pfrender & Jones 2001). Rather, it can be exactly calculated using a psychophysical model of colour

vision. This feature uniquely enables to determine the location of multiple, global and local optima and therefore to analyse both the gradual evolution of signals towards peaks and more important evolutionary transitions like peak shifts.

Last, the stimulation landscape could be extended to other senses. A necessary requirement is to obtain a lowdimensional space that describes stimuli based on their physicochemical characteristics. This requirement is met in acoustic and olfactory communication as PCA and nonlinear methods of dimensionality reduction are applicable (e.g. Ryan & Rand 2003; Guerrieri et al. 2005). A second requirement is the necessity to predict the perceptual distance between two stimuli. Currently, there is no model of perceptual space based on the peripheral sensory systems in both acoustic and olfactory perception that has the predictive power of models used in colour vision. However, multivariate perceptual spaces were accurate in predicting the perception of call attractiveness in female frogs (Rvan & Rand 2003) as well as the perceived similarity among 16 odours in honeybees (Guerrieri et al. 2005). We thus expect the stimulation landscape to be soon applicable in a variety of communication systems.

# Conclusions

In this article, we proposed a new framework to quantify and compare signal conspicuousness to multiple species. Our framework is widely applicable given that most communication systems involve perception by multiple species. Throughout this article, we assumed an equal contribution of each perceiver to the fitness of the signaller. In the real world, however, the fitness payoffs of being conspicuous to a mate versus a predator, for example, may not be the same. Research on the relative fitness payoffs of multiple perceivers is still in its infancy. Crucially, the model of stimulation landscape allows integrating relative fitness payoffs, for example, by attributing weights to individual stimulation landscapes before combining them into a synthetic landscape. We thus hope that the stimulation landscape as a general framework on communication will contribute to bridge the current gap between evolution and ecology in that field.

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#### 728 J. P. Renoult et al.

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#### **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Methods for the phylogenetically informed comparative analyses.

Fig. S1. Simulation of reflectance spectra with varying degrees of autocorrelation

Fig. S2. Reconstruction of reflectance spectra with varying number of principal components.

Fig. S3. The ecologically relevant dimensionality of the space of bird reflectance spectra

**Fig. S4**. Relation between wavelengths and wavelength coordinates for the first two components of the PCA.

Table S1. Bird species included in the study.