Animal sexual signals:
Do they maximise or optimise information content?

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signed: **Iker Vaquero-Alba**

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Abstract

Traditional models of sexual selection based on the handicap principle assume a direct linkage between the degree of sexual signal exaggeration and the bearer's quality, and set out a rather inflexible scenario where handicap exaggeration is maximised for sexual signalling purposes until it reaches the limit imposed by viability selection. Such a scenario makes it difficult to imagine the mechanisms by which multicomponent signalling systems can evolve and persist in time, given the costs of producing, disseminating and receiving signals. Based on non-equilibrium coevolutionary models, it has been suggested that variation in selection pressures derived from fluctuations in ecological and/or social conditions may lead to the emergence and maintenance of redundant and non-redundant multiple signals. Alternatively, the non-equilibrium dynamics to which coevolutionary systems are often subject can maintain multiple signals without environmental variability. Species with severe fitness constraints on costlier signal expression should be selected to utilize "cheaper" signals. And individuals not displaying at the maximum possible level might be selected to "compensate" their lack of fitness using phenotypically plastic traits, like behavioural ones. Here I investigate the effect of several potentially sexually selected barn swallow ornamental traits on several reproductive success indicators and on the habitat quality of foraging areas around breeding sites, and of several quality-indicating guppy traits on predator inspection activity, a behavioural character involved in mate choice. The findings presented here indicate ventral and throat plumage colouration, previously not studied for European barn swallows, to function as quality indicators and predict reproductive success and assortative mating patterns. Additionally, we found evidence for a "compensation mechanism" in both species studied, for individuals investing in "cheaper" sexual signals or not displaying at the maximum possible level.

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Dr. Sasha Dall, Prof. Matthew Evans and Dr. Darren Croft provided guidance for planning and structure of all experimental procedures and in preparation of the manuscript. Claire Canning, Alessandro Macario and I collected the data. I conducted the analysis and am first author on the manuscript.

Chapter Six: General discussion

The general discussion, conclusions and future prospects presented in this chapter represent my own interpretation of the data presented in the previous chapters, under the guidance of Dr. Sasha Dall and Prof. Matthew Evans.

Chapter One

Multicomponent signalling systems and the “compensation mechanism”

Traditional models of sexual selection through the handicap principle assume a direct linkage between the degree of sexual signal exaggeration and the bearers' quality (Zahavi, 1975). Likewise, they assume a rather inflexible scenario where handicap exaggeration is maximised for sexual signalling purposes, until the limit imposed by viability selection is reached. In such an scenario, it is difficult to imagine how multicomponent signalling systems can evolve and persist in time, especially when the signals involved are redundant, given there are costs associated with production, dissemination and reception of signals. These costs may manifest at the time of production and/or maintenance, and also continuously through increased risk of predation or parasitism (Magnhagen, 1991; Folstad & Karter, 1992).

Animals often experience fluctuating ecological and social conditions in their environment. The variability of such environments has important downstream consequences in a number of areas of their life, e.g. food availability, risk of predation or inter- and intraspecific competition etc. Transmission of information with sexual signalling purposes is also influenced by environmental fluctuations. Based on recent insights from non-equilibrium coevolutionary models, Bro-Jørgensen (2009), through his "fluctuating environments" hypothesis, suggested that variation in selection pressures derived from fluctuations in ecological conditions and/or social context may lead to the emergence and maintenance of both redundant and non-redundant multiple signals. When genotype-by-environment interactions (GEI) occur and environmental changes affect different genotypes in different ways, phenotypically fixed signals may become misleading, and new, more phenotypically plastic signals may evolve with a better quality-indicating value. Also, if environmental shifts make a signal too costly or too "cheap" to express for any individual, alternative condition-dependent signals, more able to reflect the variance in quality among signallers, may become selected.

Receiver preferences can also be influenced by environmental changes and these preferences themselves can act as a selective force for the evolution of alternative signals, favouring the evolution of multicomponent signalling systems. Sexual conflict over the reliability of condition-dependent indicators of genetic quality may keep the handicap process in a non-equilibrium situation (van Doorn & Weissing, 2006). Even in the absence of environmental variability the non-equilibrium dynamics, to which coevolutionary systems between signaller and receiver are often subject, can help maintain multiple signals.

Costs incurred by ornamental traits used for signalling purposes by animals can vary across a continuous range. At one end of the range, exaggeratedly expressed morphological traits, like extremely elongated tail feathers in birds, incur substantial production and/or maintenance costs, as well as increase the risk of predation via reductions in the aerodynamic and locomotive efficiency of their bearers. At the other end, behavioural signals incur significantly lower costs on a day-to-day basis, whereas pigment-based markings, still "cheaper" than the costlier morphological traits, would be located somewhere in the middle in terms of the costs they inflict to their bearers. Fitzpatrick (2000) points out that although handicap theory requires the handicap to be specific and act against the quality being signalled (Grafen, 1990; Zahavi & Zahavi, 1997), only a small proportion of Palaearctic birds have extremely elongated tails, a particularly costly ornamental trait in terms of aerodynamic efficiency present in some bird species. Species in which the day-to-day costs incurred by costlier signals have particularly severe fitness consequences might be selected to utilize "cheaper" signals such as markings to a greater extent and minimise elaboration of the more "expensive" traits.

Under non-equilibrium dynamics, not all the sexual signals may be expressed at the maximum possible level (see Saino *et al.*, 2003). This may lead to unreliable communication, if we consider the quality-indicating value of certain ornamental signals alone, and highlight the quality-indicating value of more phenotypically plastic traits (e.g. those dependent on food intake or the behavioural context) for coping with dynamic variation in selection pressures. In accordance with the "fluctuating environments" or the "sexually antagonistic coevolution" hypotheses, discussed above, species with severe fitness constraints on costlier signal expression should be selected to utilize "cheaper" signals. Furthermore, we would also expect individuals with certain genotypic characteristics and/or under certain environmental circumstances to be selected to use less costly signals. Thus, high quality individuals might not be displaying at their maximum possible level, either due to not having invested all the available resources on an "expensive" trait or as a consequence of ecological and/or social fluctuations having rendered the trait less informative, altering the original information to cost ratio. These individuals might be selected to complement their sexual display using "cheaper", phenotypically plastic signals, which might increase the amount of information signalled at a lower cost. Similarly, lower-quality individuals investing in less informative, "cheaper" traits, might be selected to "compensate" their lack of reproductive fitness using behavioural traits with implications for sexual selection. For example, European barn swallows defending higher habitat quality breeding sites (Vaquero-Alba *et al.*, unpublished data; **3**, **4**) or guppies being bolder in front of predators in the presence of females (Vaquero-Alba *et al.*, unpublished data; **5**). This "compensation mechanism", as part of new insights on multiple signalling, and unlike traditional view on costly signalling, might help explain the relatively high phenotypic variability which can be observed in certain species for traits apparently subject to a strong directional selection. Furthermore, it might contribute to explain the persistence of individuals with lower sexual display intensity in natural populations, despite the presumed selective disadvantage and an impaired reproductive fitness.

Therefore, animals may have evolved sexual signalling systems that trade off the amount of information they signal to potential mates/rivals with their costs; thus, given their individual genotypic quality, their ability to express phenotypic plasticity and the way they are affected by environmental fluctuations, such sexual signalling systems may be selected to optimise rather than maximise their information content (i.e. they should maximise their information to cost ratio).

This thesis is an experimental investigation of the hypothesized "compensation mechanism" outlined above in two key species and the implications of this for the evolution of sexually selected multicomponent signalling systems. It integrates field and lab based research on multiple signalling for the selected species, with a focus on the interactions between the sexually selected traits studied. European barn swallow, *Hirundo rustica rustica*, was chosen for field work study, as it shows a highly developed multicomponent signalling system, comprising at least two types of sexually selected ornamental traits: an "expensive" sexual signal, i.e. the extremely elongated outermost tail feathers or tail streamers, which has been subject to numerous studies of sexual selection during the last few decades, including tail length manipulations (Møller, 1994; Buchanan & Evans, 2000; Turner, 2006); and a "cheaper" signal, i.e. throat and ventral colouration patches, main quality indicator in the North American subspecies (*H. rustica erythrogaster*; Safran, 2004), although not very well studied in the nominated subspecies (but see Ninni *et al*., 2004). The Trinidadian guppy, *Poecilia reticulata*, was chosen for lab based study as it offers the possibility of exploring and experimenting with an interesting multiple signalling system which includes three different pigment-based colouration markings (melanin-based, carotenoid-based and structural) with different biochemical origins and, possibly, different production and maintenance costs, plus a sexually selected behavioural trait (predator inspection behaviour).

A methodological approach: objective plumage colour assessment

In the methodological chapter (Vaquero-Alba *et al.*, unpublished data; **2**) the technique for objectively assessing plumage colouration of barn swallows in the lab that we use along the thesis, on feathers previously collected from live birds, is calibrated by calculating its repeatability, the repeatability of measurements taken directly on live birds in the field, and the comparability across data obtained with both techniques. Although we initially follow Quesada & Senar (2006), we also apply a novel statistical method for calculating repeatabilities using GLMMs (Nakagawa & Schielzeth, 2010), which allows us to control for the confounding effect of year variance by including it in the model as a random effect. Advantages of the new method over more traditional ones are discussed.

Effect of ornamental traits on habitat quality, laying date and reproductive success

The influence of several potentially sexually selected barn swallow ornamental traits on the date of laying of the first egg and on the quality of the foraging areas around breeding sites occupied by the barn swallow pairs is investigated (Vaquero-Alba *et al.*, unpublished data; **3**). Also, tail length and ventral colouration manipulations are conducted and the influence of ornamental traits before manipulation and plumage manipulations on several reproductive success indicators is investigated (Vaquero-Alba et al., unpublished data; **4**). Sexual signalling function of pigment-based plumage markings is investigated, and evidence for supporting or rejecting the "compensation mechanism" through habitat quality hypothesis is gathered and discussed.

Compensation through predator inspection behaviour in guppies

We experimentally test the hypothesis that visually less conspicuous male guppies may compensate their lower quality-indicating value by being bolder than more conspicuous males in front of females through increased predator inspection activity (Vaquero-Alba *et al.*, unpublished data; **5**). The implications of the results for the evolution of sexual signalling systems are discussed.

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Chapter Two

Objective feather colour assessment using GLMMs and ANOVA: measurements in the lab are more reliable than in the field

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Abstract

1. Colouration in birds and other animals can act as an important sexual signal for transmitting information to rivals and potential mates. Female birds can get direct or indirect benefits by choosing to mate with elaborately ornamented males, so birds with more colourful displays often have a selective advantage. Reflectance spectrometry has become a standard tool in behavioural ecology for measuring animal colouration, especially after the discovery of tetrachromacy in birds, which includes their ability to see in the UV and thus the existence of plumage patterns invisible to humans, necessitating a reliable and objective way of assessing colouration not dependent on human vision. Measurements of plumage colouration can be taken directly on live birds in the field or in the lab, or on feathers previously collected from the animals so it is important to compare the reliabilities of these different sampling methodologies.

2. Using a spectrophotometer, we assessed the repeatability of both methods separately, as well as comparing between them, for measurements of melanin-based colouration (brightness, chroma, hue and UV chroma) in different patches of the birds' plumage. We used the ANOVA-based method for calculating the repeatability of measurements from two years separately, and the GLMM-based method to calculate the overall adjusted repeatability within each patch for data from both years. To our knowledge, this is the first time the latter, newly developed method has been used to assess the repeatabilities of colour measurements. We repeated the assessment for the whole reflectance spectrum range (300-700 nm) and only the human-visible spectrum range (400-700 nm) to assess the influence of the UV component on the reliabilities of the different plumage sampling methodologies.

3. Our results showed a very high repeatability for lab measurements and a lower but still moderate to high repeatability for field measurements, both of which

increased when limited to only the human-visible part of the reflectance spectrum, for all plumage patches except the throat, where we observed the opposite trend.

4. Repeatability between both sampling methods was quite low when including the whole spectrum in the analyses, but moderate when including only the human-visible part.

5. Our results suggest a higher reliability for measurements taken in the lab and a higher power and accuracy of the GLMM-based method. Also, they highlight the possibility of the capability to reflect UV light having different implications in different plumage patches.

Introduction

Colour vision involves the capacity to discriminate amongst different wavelengths of light independent of their intensity (Kelber *et al.*, 2003; Cuthill, 2006).

Although humans have always been attracted to the colourful displays of birds, only relatively recently have scientists appreciated the importance of a systematic understanding of both function and evolution of bird colouration, as well as the mechanisms that underpin it (Hill and McGraw, 2006).

Mating with elaborately ornamented males can provide female birds with direct benefits (if ornamental traits reflect individual condition, useful individual attributes or somatic quality independent of condition) and/or indirect benefits (good genes or attractiveness for offspring – as conspicuous and costly male traits indicate highly heritable viability) (Pomiankowski, 1987; Andersson, 1994; Garamszegi *et al.*, 2006). So, birds with more elaborated colourful displays are likely to enjoy a selective advantage, as they are often preferred as mates (Hill, 2006).

Due to the importance of studies of bird plumage colouration in behavioural and evolutionary ecology, methods for quantifying such colouration reliably and objectively are invaluable. Methods traditionally used for colour assessment include colour ranks on an arbitrary scale (Promislow *et al.*, 1992), tristimulus colour models based on human vision, like the Munsell system (Peiponen, 1992), reference colour swatches (Hill, 1990) or digital photography (Loyau *et al.*, 2005; Bortolotti *et al.*, 2006; Stevens *et al.*, 2007). All of these methods, despite being easy and affordable ways of getting colour measurements and useful in certain situations, in general lack reliability and objectivity (Andersson & Prager, 2006) , and, more importantly, are tuned to the human visual system instead of the bird visual system.

Birds do not see colours in the same way we do (Bowmaker *et al.*, 1997). They have a fourth cone type in their eyes, with a pigment that is sensitive to ultraviolet light. We are still far from understanding exactly how colours are perceived by birds (Hill and McGraw, 2006), but progress is being made towards understanding how colour vision works in general and how the spectral information is processed by birds and other non-human animals (Smith *et al.*, 2002; Kelber *et al.*, 2003; Kelber and Osorio, 2010). Indeed, methods have been developed for comparing colour patterns as birds see them, using known properties of bird eyes and generating detailed formal descriptions of colour spaces and the equations used to plot them (Endler and Mielke, 2005).

Since the 1990s, reflectance spectrometry has become the most widespread method for measuring animal colouration and a standard tool in behavioural ecology, and a wide range of methods for analyzing spectrophotometry data have emerged (Hill and McGraw, 2006). This development stems largely from the revival of interest in UV vision and tetrachromacy in birds and the fact that birds can see colours that humans cannot experience (Bennett *et al.*, 1994; see Cuthill *et al.*, 2000 for a review), with the possibility of the existence of plumage patterns invisible to the human eye, and the discovery of mate choice based on the ultraviolet part of the bird reflectance spectrum (Maier, 1994; Bennett *et al.*, 1997). Therefore, reliable and objective ways of quantifying bird coloration not dependent on human vision are at a premium at the moment. Indeed, the invention of miniature diode-array spectroradiometer systems, being lighter, more portable and affordable than previous spectrometry systems but as precise and objective in colour quantification, have provided very popular tools in any colour communication study (Hill and McGraw, 2006).

There are two main ways of assessing bird plumage colouration with spectrophotometers reported in the literature. Measurements may be taken either directly on the bird, applying the pointer of the spectrophotometer to plumage patches as they occur in situ (e.g. Senar *et al.*, 2002; Bize *et al.*, 2006; Herrera *et al.*, 2008; Catoni *et al.*, 2009; Doucet and Hill, 2009; del Cerro *et al.*, 2010), or in the lab, with feather samples collected from the field, applying the pointer to “plumage patches” created by mounting these feathers on a flat surface in a way that mimics the original plumage structure (e.g. Cuthill *et al.*, 1999; Keyser and Hill, 2000; Perrier *et al.*, 2002; Safran and McGraw, 2004; Komdeur *et al.*, 2005; McGraw *et al.*, 2005; Safran, 2007). Despite the popularity of the use of spectrophotometers for colour assessment and the growing number of studies on bird colouration, few studies have rigorously assessed the consistency of both methods for measuring the colouration of plumage patches, and the repeatability of results obtained when using either one or the other (see Quesada and Senar, 2006 for such a comparison for carotenoid-based plumage coloration in great tits).

In contrast, there is little consensus about how to quantify the reliability, or repeatability, of spectral measurements. The most common measure of repeatability, or more precisely the coefficient of intraclass correlation (r_i), can be formally defined as the proportion of the total variance explained by differences among groups (Nakagawa and Schielzeth, 2010; Sokal and Rohlf, 1995):

$$r_i = \sigma_\alpha^2 / \sigma_\alpha^2 + \sigma_\varepsilon^2 \qquad \text{(eqn 1)}$$

where σ_α^2 is the between-group variance and σ_ε^2 the within-group variance, whereas the sum of both comprises the total phenotypic variance (Nakagawa and Schielzeth, 2010). Until recently, the most common ways to estimate repeatabilities from data with Gaussian errors have employed the correlation-based method (Sokal and Rohlf, 1995) or the ANOVA-based method, commonly used by behavioural and evolutionary ecologists (Donner, 1986; see Lessells and Boag, 1987 for a complete review and description). However, Nakagawa and Schielzeth (2010) describe an innovative method for calculating GLMM-based repeatability estimates, which allows for confounding variables to be factored out and calculates the confidence intervals for each repeatability calculation, inferred from distributions of repeatabilities obtained by parametric bootstrapping.

In this study, using both ANOVA-based and GLMM-based methods, we test the consistency of measuring melanin-based plumage colouration in four different patches of the ventral plumage of the European barn swallow *Hirundo rustica rustica*, both directly on the bird and on feather samples in the laboratory, and we determine the repeatability of the results obtained with both methods. To our knowledge, this is the first time GLMM-based repeatability estimates have been used to assess the reliability of melanin-based plumage colouration measurements.

Materials and methods

Field work was carried out during May-August 2009 and March-August 2010 in several sites, mainly farms, located in the surroundings of the University of Exeter Campus, in Cornwall, UK (Fig. 1). 59 adult European barn swallows, *Hirundo rustica rustica* (21 in 2009, 38 in 2010)*,* were caught using mist nets, ringed, morphometric measurements taken, plumage reflectance spectra quantified in the field and feather samples collected for subsequent assessment in the lab.

Colour was quantified based on Endler & Mielke (2005), using a USB2000 spectrometer (Ocean Optics, Dunedin, Florida), and a xenon flash lamp (Ocean Optics). We used a WS-1 SS Diffuse Reflectance Standard, a diffuse white plastic >98% reflective from 250-1500 nm, as the white reference (100% reflectance), and a piece of black velvet as the dark standard (0% reflectance) to correct for the noise when no light is reaching the sensor. At the far end of the reflection probe/light source, we put a non-reflective black pointer cut in a 45 degree angle, to avoid mismeasurement derived from the light reflected by the plumage reaching the sensor (Andersson and Prager, 2006; Pike, pers. comm.). Using the spectra acquisition software package OOIBase (Ocean Optics), we measured the reflectance of four body regions, namely the throat, breast, belly and vent of each bird (Fig. 2). For the measurements of feather samples in the lab, we collected enough feathers from live birds as to being able to mount them one on top of the other and simulate the original pattern found on live birds. We mounted the feathers on a piece of black velvet to avoid background noise. Once we had tested for the reliability of both methods separately, we averaged the three measurements for each method and used these average values to test the comparability between field and lab measurements.

We used the method described in Endler and Mielke (2005) to calculate brightness, chroma and hue, parameters generally used to specify a colour. Using their equations and the mathematical software Matlab (The MathWorks Inc., Natick, MA), we got the spectral sensitivity functions of the cones corrected for the cut points of oil droplets, calculated the quantal catch for each photoreceptor and converted those quantal catches into dimensional colour space coordinates in a tetrahedral colour space (Fig. 3). Chroma is defined as the strength of the colour signal or the degree of difference in stimulation among the cones, and it is proportional to the Euclidean distance from the origin, that is, the distance from the bird grey (achromatic) point to each point, specified by three space coordinates. Perception of hue depends of which cones are stimulated, and in tetrahedral colour space, it is defined by the angle that a point makes with the origin. As bird colour space is 3D, hue is defined by two angles, analogous to latitude and longitude in geography (Endler and Mielke, 2005).

Brightness is defined as the summed mean reflectance across the entire spectral range ($R_{300–700}$; Montgomerie, 2006; Galván and Møller, 2009). As well as these parameters, we included UV chroma, a measure of spectral purity, into our analysis, which was calculated as the proportion of reflectance in the UV part of the spectrum ($R_{300-400}$) in relation to the total reflectance spectrum ($R_{300–700}$; Siefferman and Hill, 2005). Cone sensitivities and oil droplet cut points were taken from Bowmaker *et al.* (1997), Hart (2001), Vorovyev *et al.* (1998), Govardovskii *et al.* (2000) and Hart and Vorovyev (2005).

Although all the avian families investigated show plumages reflecting significant amounts of UV light (see Eaton and Lanyon, 2003 for a review), in the particular case of barn swallows, ventral plumage shows a noisy reflectance pattern in the UV part of the spectrum, with some values even below 0, and does not exhibit a clear ultraviolet reflectance peak (Fig. 4; Safran and McGraw, 2004). For this reason, we calculated the same colour variables both including and not including the UV part of the reflectance spectrum, and carried out a repeatability assessment using either the whole reflectance spectrum or only the visible part. When using only the visible part, we did not include UV chroma, for obvious reasons, nor hue, as values are identical in both cases.

ANOVA-based method: In order to test for the reliability of both procedures separately, we calculated the repeatability for colour variables in the four patches for the different procedures according to Lessells and Boag (1987), Senar (1999) and Quesada and Senar (2006). Repeatabilities were computed from the mean squares of ANOVA on three repeated measures per individual. Both in field and lab procedures, the second and third measurements were made after removing the reflection probe/light source and placing it again on the colouration patch. IV took all the measurements.

Once we calculated the repeatabilities for each method, we averaged the three measurements per patch per individual and assessed the repeatability of measurements across procedures, but this time the ANOVA was carried out on two repeated measures per individual, one from the field and another one from the lab.

We repeated this process for both 2009 and 2010 data separately. Because of the particular circumstances in which data were collected each year (Vaquero-Alba, Evans & Dall, unpublished), colour measurements in 2009 were taken in three different points within each patch, whereas in 2010 the three of them were taken in the same point.

GLMM-based method: We used a modified version of the R function *R.Anson*, which is itself a modification of rpt.remlLMM function (Nakagawa and Schielzeth, 2010). We fitted two random-effect terms (individual identity and year) in our linear mixed-effects models, and calculated the adjusted repeatability estimate as:

$$r_i = \sigma_\alpha^{\,2} / \sigma_\alpha^{\,2} + \sigma_\varepsilon^{\,2} + \sigma_r^{\,2} \qquad \text{(eqn 2)}$$

where $\sigma_r^{\,2}$ is the year variance.

In order to have a general idea of repeatability for each patch, we included all the colour variables in a principal component analysis (PCA) and calculated the repeatability (and confidence intervals) for the first component (PC1) within each plumage patch.

As we conducted multiple statistical tests on data subsets that are not likely to be biologically independent of each other (i.e. different components of the spectra, same metrics on different years, or same metrics in the lab and in the field), there was an increased probability of type I error rates. To control for this increased probability, we corrected our p-values for multiple testing based on the sequentially rejective Bonferroni procedure of Holm (Holm, 1979), using the *stats::p.adjust* function in R (R Development Core Team, 2010).

All the statistical analyses were carried out using R (Crawley, 2007; R Development Core Team, 2010).

Results

ANOVA: **2009**

When including the whole spectrum in the analyses, measuring plumage colouration in the lab proved to be a reliable method, with brightness, UV chroma, chroma and hue latitude and longitude being highly repeatable for all the patches, and hue latitude in the throat being less repeatable (r_i=0.418, $F_{21,44}$=3.157, p=0.01). The method of measuring the plumage colouration in the field was also quite consistent but with overall lower values of repeatability, although still reasonably high, for all the variables and patches, being especially low for hue latitude in breast (r_i=0.382, $F_{20,42}$=2.856, p=0.025) and vent (r_i=0.394, $F_{21,44}$=2.955, p=0.017; Table 1).

When including only the visible part of the spectrum in the analysis, overall repeatability was lower. The lab method again proved to be the most reliable, with high values of repeatability for brightness and chroma in all the patches. The field procedure was moderately repeatable for belly and throat, but showed low repeatability for brightness in the breast (r_i=0.36, $F_{20,42}$=2.6885, p=0.038) and for chroma in the vent (r_i=0.428, $F_{21,44}$=3.241, p=0.007; Table 2).

The values of repeatability across the field and laboratory procedures were very low for all the patches measured, both considering the whole reflectance spectrum or only the visible part (r_i<0.35 and p>0.05 in all cases), suggesting a lack of consistency across the two assessment methods for melanin-based plumage colouration. Repeatabilities of brightness measurements were slightly higher for the whole spectrum than for only the visible part, in all the patches except from the vent. However, in this case, including only the visible part of the spectrum yielded much more repeatable chroma values than including the whole range, sometimes even turning negative repeatability values into positive, e.g. for the belly (whole range: r_i=-0.507, $F_{20,21}$=0.327, p=1; only visible range: r_i=0.346, $F_{20,21}$=2.056, p=0.599), or the vent (whole range: r_i=-0.758, $F_{21,22}$=0.138, p=1; only visible range: r_i=0.296, $F_{21,22}$=1.842, p=0.657; Table 1 and Table 2).

ANOVA: **2010**

When including the whole spectrum in the analyses, repeatability measurements in the field yielded considerably higher results than in 2009, with all the r_i values above 0.60, except from hue latitude in the throat (r_i=0.515, $F_{37,75}$=4.186, p<0.0001), and with most of the values ranging from 0.74 to 0.91, except for brightness in the breast (r_i=0.611, $F_{37,76}$=5.710, p<0.0001), hue latitude in the belly (r_i=0.63, $F_{37,76}$=6.1, p<0.0001), hue latitude in the vent (r_i=0.629, $F_{37,76}$=6.088, p<0.0001) and hue longitude in the vent (r_i=0.679, $F_{37,76}$=7.356, p<0.0001). In the lab, repeatability values ranged from 0.71 to 0.95 in most of the patches, except from hue latitude in the throat (r_i=0.65, $F_{37,76}$=6.569, p<0.0001), and repeatability was overall higher than when measuring it on live birds, except from UV chroma in the belly (r_i=0.857, $F_{37,76}$=18.986, p<0.0001), breast (r_i=0.788, $F_{37,76}$=12.117, p<0.0001) and vent (r_i=0.819, $F_{37,76}$=14.571, p<0.0001) and hue latitude in the breast (r_i=0.722, $F_{37,76}$=8.808, p<0.0001), where it was slightly lower. Repeatability values were similar to the ones obtained in 2009 (Table 3).

When doing the analysis including only the visible part of the spectrum, measuring colouration in the lab was again the most reliable method of both, with all the r_i values above 0.91, except for chroma in the throat (r_i=0.881, $F_{37,76}$=22.964, p<0.0001). Field procedure still yielded high repeatability values, with brightness in the breast (r_i=0.569, $F_{37,76}$=4.959, p<0.0001) and chroma in the vent (r_i=0.696, $F_{37,76}$=7.839, p<0.0001) being the only measurements with values below 0.81. For both methods, repeatability values were higher than in 2009 for all the variables within all the plumage patches (Table 4).

Repeatabilities across field and lab methods in 2010 were quite heterogeneous including the whole spectrum in the analyses: moderately high for hue longitude in the belly (r_i=0.794, $F_{37,38}$=8.732, p<0.0001) and breast (r_i=0.657, $F_{37,38}$=4.818,

$p<0.0001$), moderate for vent hue latitude ($r_i=0.463$, $F_{37,38}=2.723$, $p=0.018$) and longitude ($r_i=0.561$, $F_{37,38}=3.553$, $p=0.001$), belly hue latitude ($r_i=0.431$, $F_{37,38}=2.515$, $p=0.034$) and breast brightness ($r_i=0.482$, $F_{37,38}=2.861$, $p=0.012$), and low for breast chroma ($r_i=0.326$, $F_{37,38}=1.966$, $p=0.21$), throat UV chroma ($r_i=0.321$, $F_{37,38}=1.944$, $p=0.312$) and chroma ($r_i=0.274$, $F_{37,38}=1.755$, $p=0.576$) and vent brightness ($r_i=0.349$, $F_{37,38}=2.070$, $p=0.141$). For the rest of the cases, repeatabilities were very low ($r_i<0.23$ and $p>0.05$ for all the cases). When including only the visible part of the spectrum, repeatability was moderate to high and had a significant effect for both brightness and chroma in the breast, and for chroma in the vent and in the belly, whereas it was quite low and non-significant for brightness in the belly ($r_i=0.217$, $F_{37,38}=1.556$, $p=0.899$), and very low for both variables in the throat. Repeatability values for chroma in all the patches except for the throat were much higher than when we included the whole spectrum range, e.g. in the belly (whole range: $r_i=-0.049.$, $F_{37,38}=0.906$, $p=1$; only visible range: $r_i=0.75$, $F_{37,38}=7.005$, $p<0.0001$) and in the vent (whole range: $r_i=0.224$, $F_{37,38}=1.579$, $p=0.657$; only visible range: $r_i=0.416$, $F_{37,38}=2.428$, $p=0.047$; Table 3 and Table 4).

GLMM: **2009+2010**

When we included the whole spectrum in the analyses, for all the principal component analyses carried out within each patch for field and lab measurements, PC1 accounted for more than a 53% of the total variance, except for vent measurements in the field (where it explained a 49% of the total variance) and for measurements in the throat (where it explained between 44% and 47%). When including only the visible part of the spectrum, PC1 explained a 67% of the total variance for vent measurements in the field, and between 75% and 93% in the rest of the cases.

Repeatability of feather colour measurements was much higher and confidence intervals smaller when quantifying colouration in the lab than when doing it on live birds in the field, both including the whole spectrum in the analyses or only the visible part, being particularly high in the breast (whole range: r_i=0.916, 95%CI=[0.855,0.943], $p<0.0001$; visible range: r_i=0.927, 95%CI=[0.872,0.95], $p<0.0001$). All the repeatability values from lab measurements ranged between 0.71 and 0.93 and were highly significant ($p<0.0001$).

When using field measurements, repeatabilities were still moderately high (all r_i values above 0.50) and higher when including only the visible spectrum range in the analyses than including the whole range, except in the throat (whole range: r_i=0.629, 95%CI=[0.303,0.874], $p<0.0001$; visible range: r_i=0.564, 95%CI=[0.266,0.816], $p<0.0001$; Fig. 5).

Repeatabilities across both field and lab methods yielded higher results when we included only the visible spectrum in the analyses than when we included the whole spectrum. Leaving the values for throat apart, as here repeatability was not significantly different from zero no matter the spectrum we included in the analyses, r_i ranged between 0.19 and 0.41 when including the whole spectrum, and between 0.44 and 0.62 when including only the visible part (Fig.5). All the repeatabilities in belly, breast and vent were significant except for the belly when including the whole spectrum (r_i=0.189, 95%CI=[0,0.415], $p=0.069$), but it became significant and higher when only the visible spectrum was included in the analyses (r_i=0.503, 95%CI=[0.281,0.667], $p<0.0001$, Fig. 5).

Discussion

Measuring plumage colouration from feather samples in the lab proved to be a highly reliable method, with high values of repeatability in general for all the variables and patches in 2009, 2010 and when applying the GLMM-based method for both years. Measuring colouration directly on bird plumage in the field was also reliable, but with overall lower values of repeatability than the lab for most of the variables measured on different patches, with the exception of some variables in the throat in 2009 and UV chroma measurements in the belly, breast and vent in 2010 when considering the whole spectrum, which yielded higher values of repeatability when measured in the field. This may be due, on the one hand, to the fact that throat patch is smaller and much darker than the rest of the patches and the feathers that constitute it much smaller, so it is often quite difficult to obtain a reliable reflectance measurement with such a limited amount of photons reaching the spectrophotometer probe. Also, it is more difficult to create a “plumage patch” in the lab with a feather arrangement similar to the bird’s original one and big enough to be able to apply the spectrophotometer pointer to it. On the other hand, UV part of the spectrum shows a very noisy pattern in this species, so we would not necessarily expect highly consistent UV chroma repeatabilities across field or lab measurements, which may explain why repeatability values in the lab for UV chroma measurements are higher than in the field in 2010, whereas the rest of the repeatability values tend to be higher in the lab.

So, collecting feathers from birds and assessing their colouration in the lab, as well as being more convenient, minimising risk to a sensitive device like a spectrophotometer and reducing handling times of the animals (Quesada and Senar, 2006), is a more reliable method for assessing melanin plumage colouration than doing so directly on live birds, according to our results.

When comparing both methods, the values obtained in 2009 for different variables measured in different plumage patches directly on the bird were poorly repeatable compared to the values obtained for the same variables measured from feather samples, and non-significant in all cases. In 2010, in contrast, repeatabilities were higher and significant for certain metrics in certain patches only. These results stand in marked contrast to the positive results of another study, which compared the repeatabilities between both colouration assessment procedures for carotenoid-based plumage (Quesada and Senar, 2006). There can be several reasons for this difference: first of all, due to the different characteristics of the two types of pigments, carotenoid-derived colouration is more variable among individuals than melanin-based colouration (Badyaev and Hill, 2000), and repeatability of a character increases with variability (Senar 1999). In order to increase the repeatability of some measurements, a possible solution could be to increase the number of measurements, for example from three to five, as it has already been done by several authors (e.g. Bennett *et al.*, 1997; Perrier *et al.*, 2002; Doucet and Hill, 2009). Unfortunately, this is not an option when working with live birds in the field, as we would be increasing the manipulation times and, consequently, the stress levels to an unacceptable degree, although it can be applied when assessing colouration in the lab on feather samples (Quesada and Senar, 2006).

Due to the way data were collected, the three plumage colouration measurements taken in the field in 2009 covered a wider area of each plumage patch than the measurements made on feather samples, which were restricted to the area covered by the bunch of feathers plucked from each patch on each individual. For that reason, repeatability of 2009 field measurements can be taken as an estimate of colouration consistency within the plumage patches. Our results suggest a moderate to high within-patch consistency for melanin-based ventral colouration in the European barn swallow *Hirundo rustica rustica*. In 2010, however, the three field measurements were taken approximately in the same plumage area for each patch. As a result of this, the comparability of both procedures in 2009 may have been compromised, although the repeatability of the 2010 samples was higher even for lab measurements, especially when considering only the visible part of the spectrum, which may be indicative of higher patch colouration homogeneity in 2010.

The GLMM-based method (Nakagawa and Schielzeth, 2010), applied to data from both years, allowed us to control for year effects by adding the year variance into the total variance calculation , so that we could obtain the adjusted repeatability for data from both years. Thanks to the PCA, which allowed us to create composite variables accounting for almost 50% of the total variance in the metrics taken from each patch, we were able to estimate the overall repeatability within each patch for both methods separately and across methods. The possibility of calculating adjusted repeatabilities by including year as a random factor, together with the reduction in the number of variables accounting for a great proportion of the total variance achieved by the PCA, considerably reduced the amount of multiple tests necessary for repeatability calculation. Thus, the p-values obtained with this method were less affected by Bonferroni corrections than those obtained with the ANOVA-based method, reducing the probability of type II errors and increasing the power of this method for repeatability calculation. Repeatability was moderate to high within all the patches for field measurements, and considerably higher for lab measurements, suggesting that lab method is a more reliable way of assessing melanin-based colouration. The fact that almost all the repeatability measurements, and especially the repeatabilities across field and lab methods (in patches other than the throat), were higher when including only the human-visible spectrum in the analyses , suggests that the noisy reflectance pattern in the UV part of the spectrum may be distorting the results and underestimating the comparability of the two methods. For throat plumage, however, we observed the opposite trend, with higher repeatability values when including the whole spectrum, which could be indicative that the UV part of the spectrum is more important in the throat than in the rest of the patches. We find support for this idea when looking at reflectance spectra plots for different patches (Fig. 4): throat reflectance spectra, although showing also quite a noisy pattern for the UV part, and unlike the rest of the patches' spectra, tends to show UV reflectance peaks in both sexes. Further work is needed to find out whether there are UV reflectance differences amongst different plumage patches.

In conclusion, our results suggest that collecting feathers from live animals and assessing colouration in the lab is more reliable than taking measures on live birds directly, in the field. Moreover, since it is easier on equipment and minimises the length of time birds need to be handled (minimising the stress levels inflicted on them), feather sampling would appear to be the best method available. We also demonstrated the value of the GLMM-based method (Nakagawa and Schielzeth, 2010) for repeatability calculation, as it enables random factors to be accounted for and can calculate adjusted repeatability values, which are more accurate that those calculated using other (e.g. ANOVA) methods and increase the power of the tests. Finally, we have also shown that it is important to check for the effect that the UV part of the spectrum could be exerting on repeatability calculations, as the capability of the plumage to reflect the UV light could have different biological implications in different plumage patches.

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	UV+Visible range							
	Belly		Breast		Throat		Vent	
	F	r_i	F	r_i	F	r_i	F	r_i
Repeatability field								
Brightness	6.0446	__0.627***__	3.1183	__0.414*__	12.273	__0.79***__	6.1868	__0.634***__
UV Chroma	10.203	__0.754***__	14.171	__0.814***__	13.861	__0.811***__	5.3196	__0.59***__
Chroma	9.0161	__0.728***__	11.238	__0.773***__	10.714	__0.764***__	4.5611	__0.543***__
Hue latitude	4.0921	__0.508***__	2.8564	__0.382*__	4.4146	__0.532***__	2.9545	__0.394*__
Hue longitude	13.025	__0.8***__	9.1422	__0.731***__	24.348	__0.886***__	4.6771	__0.55***__
Repeatability lab								
Brightness	13.188	__0.802***__	14.777	__0.821***__	8.2092	__0.706***__	20.212	__0.865***__
UV Chroma	46.493	__0.938***__	23.015	__0.88***__	34.895	__0.919***__	25.561	__0.892***__
Chroma	42.489	__0.932***__	26.975	__0.896***__	62.481	__0.954***__	29.052	__0.903***__
Hue latitude	11.986	__0.785***__	6.5101	__0.647***__	3.1573	__0.418**__	23.024	__0.88***__
Hue longitude	25.986	__0.893***__	9.2833	__0.734***__	6.0119	__0.625***__	27.291	__0.898***__
Comparison field-lab								
Brightness	1.4636	**0.188**	2.0702	**0.349**	0.865	**-0.072**	1.8043	**0.287**
UV Chroma	1.3153	**0.136**	1.3591	**0.152**	0.7499	**-0.143**	0.9006	**-0.052**
Chroma	0.327	**-0.507**	1.3715	**0.157**	1.0143	**0.007**	0.1376	**-0.758**
Hue latitude	0.9084	**-0.048**	0.8635	**-0.073**	1.2523	**0.112**	0.706	**-0.172**
Hue longitude	1.7364	**0.269**	1.7513	**0.273**	0.6709	**-0.197**	1.1055	**0.05**

‘***’ p<0.001; ‘**’ p<0.01; ‘*’ p<0.05 ‘.’ p<0.1

Table 1: ANOVA-derived Repeatabilities in 2009 plumage colouration measurements taken from live birds in the field, feather samples in the lab, and across both procedures (UV+Visible spectrum).

	Visible range							
	Belly		Breast		Throat		Vent	
	F	**r_i**	**F**	**r_i**	**F**	**r_i**	**F**	**r_i**
Repeatability field								
Brightness	4.8958	**0.565*****	2.6885	**0.36***	11.221	**0.773*****	5.6072	**0.606*****
Chroma	6.7724	**0.658*****	6.4868	**0.646*****	6.6207	**0.652*****	3.2416	**0.428****
Repeatability lab								
Brightness	11.818	**0.783*****	15.892	**0.832*****	6.9936	**0.666*****	19.326	**0.859*****
Chroma	28.865	**0.903*****	21.117	**0.821*****	29.402	**0.873*****	23.838	**0.901*****
Comparison field-lab								
Brightness	1.461	**0.187**	2.0097	**0.335**	0.6804	**-0.19**	1.8902	**0.308**
Chroma	2.0563	**0.346**	1.6683	**0.25**	1.1396	**0.065**	1.8415	**0.296**

'***' p<0.001; '**' p<0.01; '*' p<0.05 '.' p<0.

Table 2: ANOVA-derived Repeatabilities in 2009 plumage colouration measurements taken from live birds in the field, feather samples in the lab, and across both procedures (Only Visible part of the spectrum).

	UV+Visible range							
	Belly		Breast		Throat		Vent	
	F	r_i	F	r_i	F	r_i	F	r_i
Repeatability field								
Brightness	16.422	**0.837*** **	5.7104	**0.611*** **	17.398	**0.846*** **	14.534	**0.819*** **
UV Chroma	25.333	**0.89*** **	15.853	**0.832*** **	12.357	**0.791*** **	16.829	**0.841*** **
Chroma	24.037	**0.885*** **	14.749	**0.821*** **	22.212	**0.876*** **	12.377	**0.792*** **
Hue latitude	6.0997	**0.630*** **	9.7411	**0.744*** **	4.1857	**0.515*** **	6.0878	**0.629*** **
Hue longitude	23.669	**0.883*** **	31.363	**0.910*** **	9.8914	**0.748*** **	7.3556	**0.679*** **
Repeatability lab								
Brightness	55.197	**0.947*** **	31.387	**0.910*** **	30.9	**0.909*** **	44.036	**0.934*** **
UV Chroma	18.986	**0.857*** **	12.117	**0.788*** **	17.544	**0.847*** **	14.571	**0.819*** **
Chroma	25.375	**0.890*** **	25.936	**0.893*** **	27.679	**0.899*** **	39.854	**0.928*** **
Hue latitude	8.3908	**0.711*** **	8.8076	**0.722*** **	6.5692	**0.650*** **	9.1422	**0.731*** **
Hue longitude	37.357	**0.924*** **	31.683	**0.911*** **	11.664	**0.781*** **	37.517	**0.924*** **
Comparison field-lab								
Brightness	1.3041	**0.131948**	2.8612	**0.482***	0.7642	**-0.134**	2.0703	**0.349**
UV Chroma	0.5572	**-0.284**	1.059	**0.029**	1.9441	**0.321**	1.5873	**0.227**
Chroma	0.9059	**-0.049**	1.9662	**0.326**	1.755	**0.274**	1.5788	**0.224**
Hue latitude	2.5152	**0.431***	1.2479	**0.110243**	0.7839	**-0.121**	2.7232	**0.463***
Hue longitude	8.7322	**0.794*** **	4.8175	**0.657*** **	**0.9731**	**-0.014**	3.5525	**0.561** **

‘***’ p<0.001; ‘**’ p<0.01; ‘*’ p<0.05 ‘.’ p<0.1

Table 3: ANOVA-derived Repeatabilities in 2010 plumage colouration measurements taken from live birds in the field, feather samples in the lab, and across both procedures (UV+Visible spectrum).

	Visible range							
	Belly		Breast		Throat		Vent	
	F	r_i	F	r_i	F	r_i	F	r_i
Repeatability field								
Brightness	14.408	0.817***	4.959	0.569***	16.59	0.839***	13.527	0.806***
Chroma	22.566	0.878***	22.163	0.876***	20.007	0.864***	7.8386	0.696***
Repeatability lab								
Brightness	59.713	0.951***	34.778	0.918***	34.133	0.917***	49.55	0.942***
Chroma	51.455	0.944***	57.897	0.950***	22.964	0.881***	72.314	0.959***
Comparison field-lab								
Brightness	1.5559	0.217	3.1819	0.522**	0.679	-0.191	2.3696	0.406.
Chroma	7.0047	0.750***	7.0261	0.751***	1.0876	0.042	2.4275	0.416*

‘***’ p<0.001; ‘**’ p<0.01; ‘*’ p<0.05 ‘.’ p<0.1

Table 4: ANOVA-derived Repeatabilities in 2010 plumage colouration measurements taken from live birds in the field, feather samples in the lab, and across both procedures (Only Visible part of the spectrum).

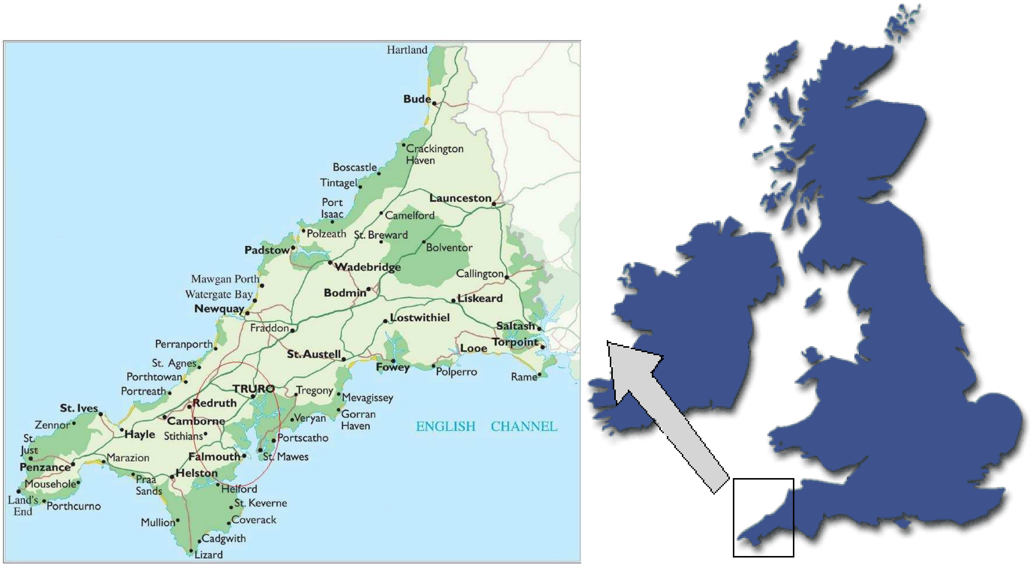

Figure 1: Location of the sampling area.

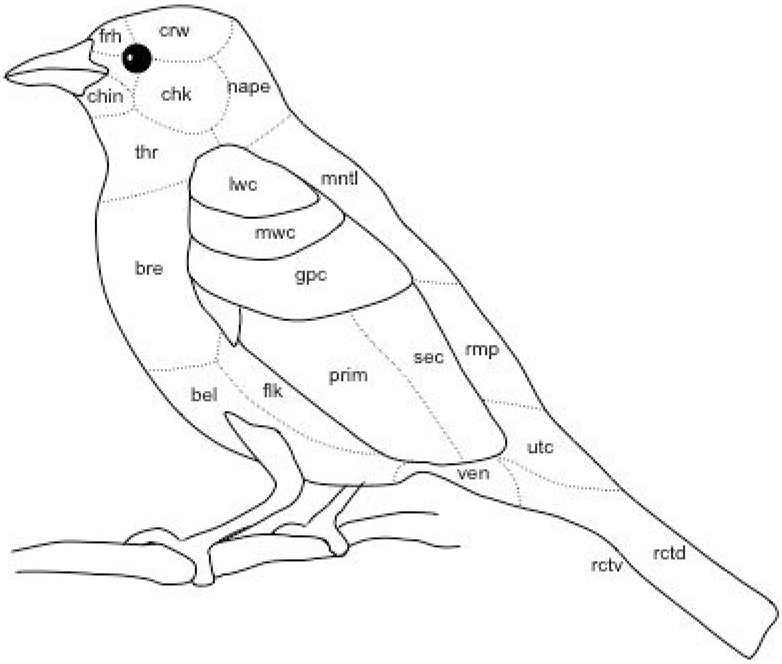

Figure 2: Topography of bird plumage regions: *thr* throat, *bre* breast, *bel* belly, *ven* vent (modified from Andersson & Prager, 2006).

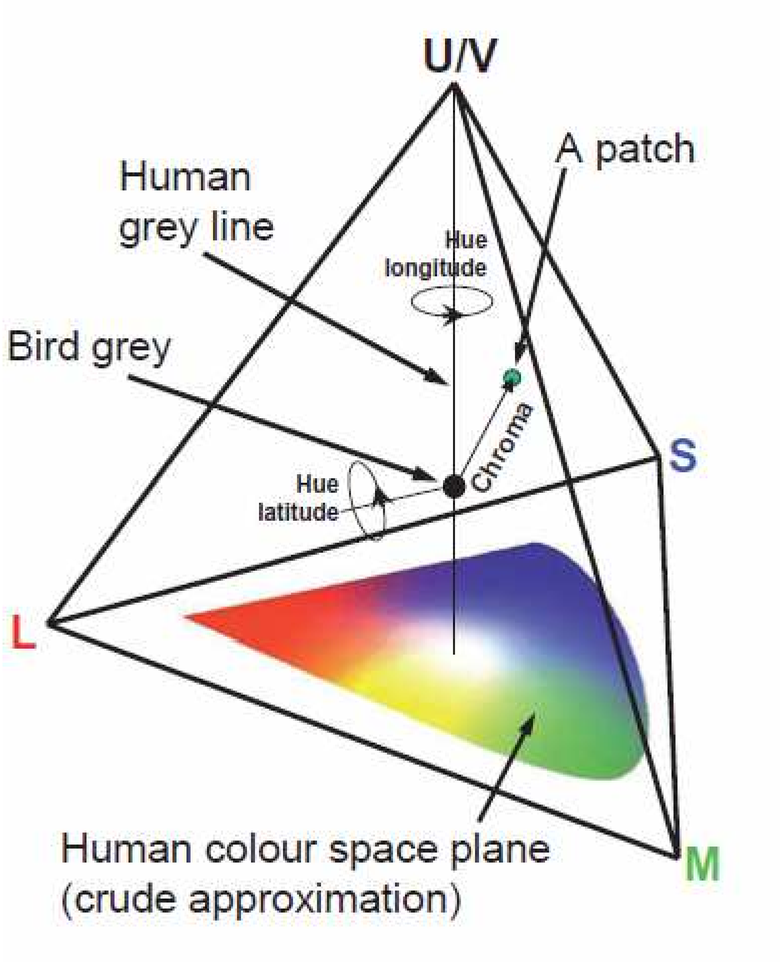

Figure 3: The avian tetrahedral colour space (from Endler & Mielke, 2005).

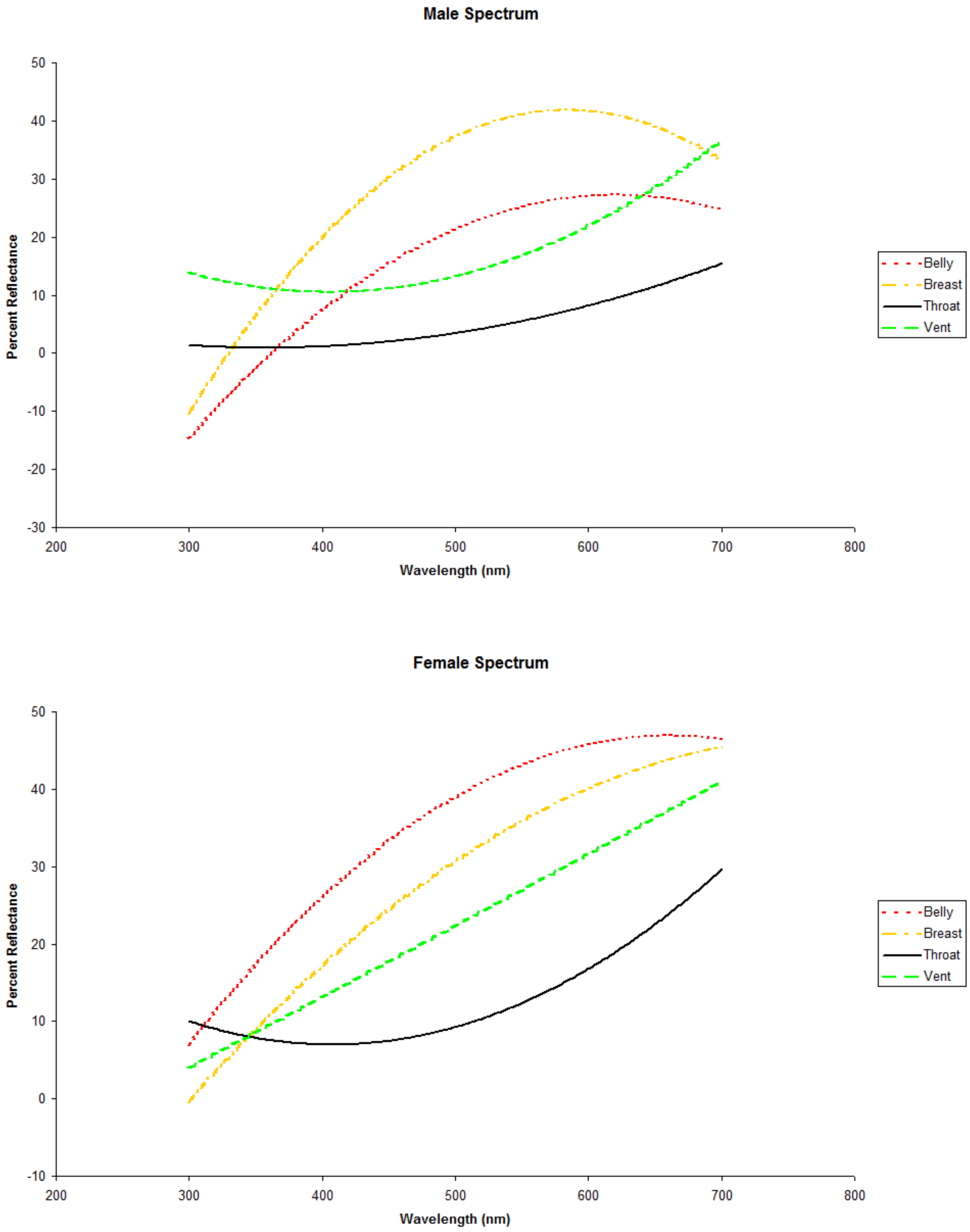

Figure 4: Reflectance spectra for belly, breast, throat and vent patches of male and female barn swallow.

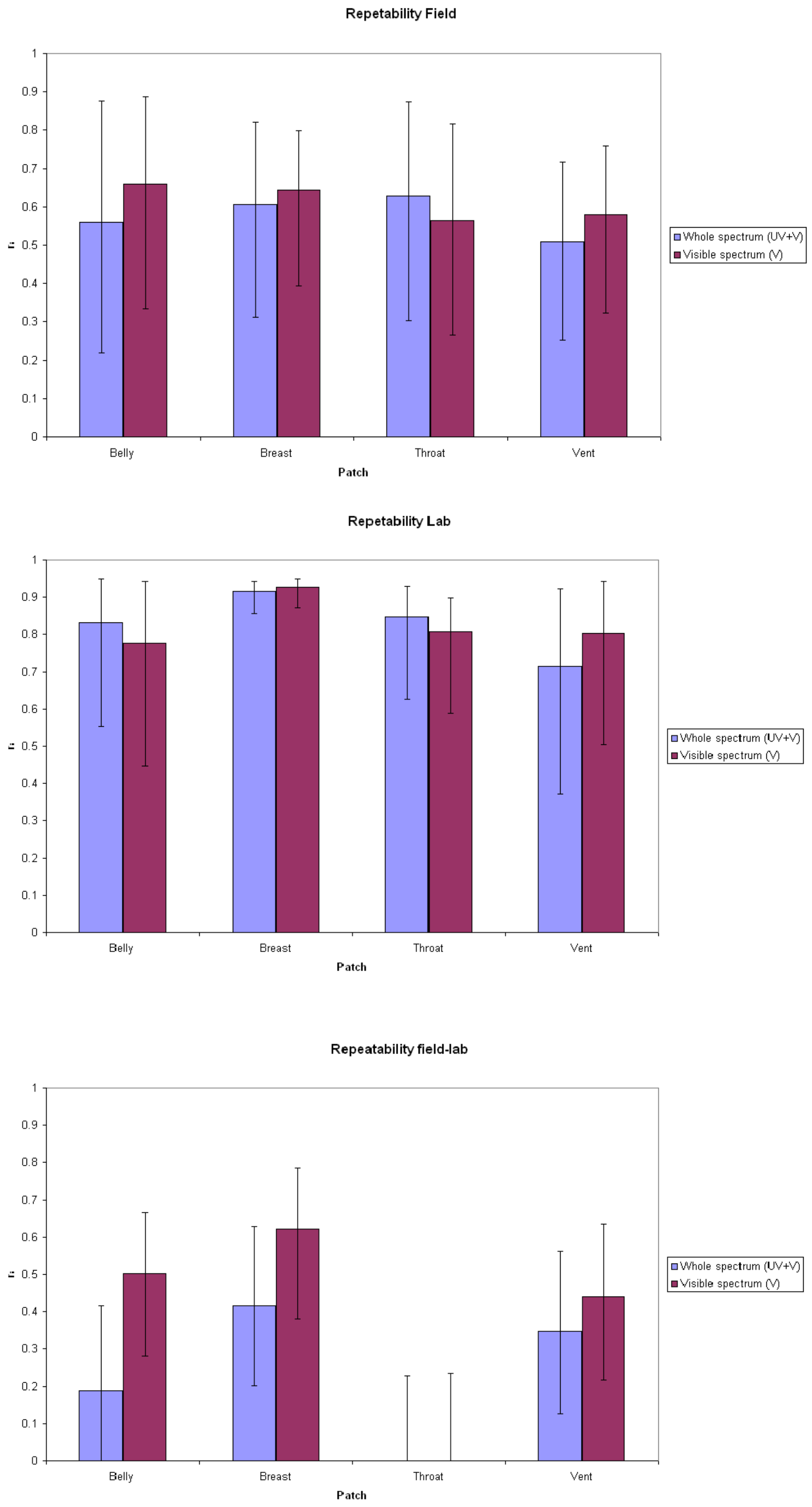

Figure 5: Repeatabilities (± 95% CI) in 2009-2010 plumage colouration measurements taken a) from live birds in the field, b) from feather samples in the lab, and c) across both procedures, both when including the whole light spectrum or only the human-visible spectrum in the analyses.

Chapter Three

Multiple signalling, habitat quality and laying date in the European barn swallow *Hirundo rustica rustica*

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Abstract

Mating with elaborately ornamented males can provide female birds with direct and/or indirect benefits. Variation in selection pressures derived from fluctuations in the environment (ecological and social context) leads animals to use more than one signal to transmit information to others, in spite of the costs associated. We tested this idea, using the European barn swallow as our model species. We hypothesized that males arriving later to the breeding grounds should be selected to decrease the investment in tail streamer elongation in favour of "cheaper" ornaments, in order to balance the information transmitted and the costs associated. Also, we propose a "compensation mechanism" for individuals with a higher elaboration of "cheaper" sexual signals, according to which they should be selected to defend breeding sites associated with a higher quality foraging area, to outweigh their lack of fitness. As we predicted, individuals with darker throat and ventral plumage patches, or those with either brighter patches or in poor condition, occupied nesting sites next to areas richer in large insects. Also, pairs with individuals in better physical condition, and those with darker-throated males, started laying earlier. Plumage colouration seemed to be a more important sexual signal in our study population than previously thought for European barn swallow, suggesting a differential adaptation to dynamic selection pressures amongst populations.

Introduction

Mating with elaborately ornamented males can provide female birds with direct or indirect benefits, information about which is transmitted by inter-individual differences in the degree of exaggeration of the ornaments (Pomiankowski, 1987; Andersson, 1994; Garamsziegi *et al.*, 2006). So, birds with more elaborate, colourful displays enjoy a selective advantage, as they are preferred as mates (Hill, 2006).

Such ornamental traits are also present in females of several species, and males can also benefit from mating with elaborately ornamented females, given that female ornamentation provides information on female reproductive capacity: older, more ornamented females breed earlier and lay larger clutches, so they can fledge more young (Komdeur *et al.*, 2005). Thus, males and females do necessarily not gain fitness advantages from choosing mates with elaborate plumage in the same way (Komdeur *et al.*, 2005).

Elaborate and colourful displays used for signalling purposes by birds are costly at evolutionary stability, according to models of sexual selection (Fisher, 1930; Zahavi, 1975; Iwasa *et al.*, 1991). Costs are expressed during production and/or maintenance, and continuously through increased risk of predation or parasitism (Magnhagen, 1991; Folstad & Karter, 1992). More “expensive” signals, like elongated tail feathers, incur substantial production/maintenance costs and increase the risk of predation via reductions in the aerodynamic efficiency of their bearers. Other, “cheaper” signals, such as pigment-based plumage markings, or especially behavioural traits, incur substantially lower costs, particularly on a day-to-day basis. In the continuum of costs incurred and information transmitted by sexual signals, we would expect higher-quality individuals to benefit from using “expensive” signals such as tail elongation: for handicap signalling to work as an ESS, marginally better individuals should have larger marginal fitness effects of advertising, because of lower costs, higher benefits or both (Getty, 1998).

Nevertheless, only a small proportion of Palaearctic birds have extremely elongated tails. The marginal viability cost of advertising may increase with quality as long as the higher viability costs are compensated by higher fecundity benefits (Getty, 1998). Thus, in species in which the day-to-day costs incurred by elongated tails have particularly severe fitness consequences, or in circumstances in which it does not compensate to signal at maximal intensity (e.g. at low population densities when females cannot be as choosy), signalling individuals might be selected to utilize "cheaper" signals such as markings to a greater extent and minimise elaboration of the more "expensive" traits such as tail elongation (Fitzpatrick, 2000). Therefore, birds may have evolved plumage that trades off the amount of information it signals to potential mates/rivals with its costs; in other words, such traits may be selected to optimise rather than maximise their information content.

Animals often use more than one signal to transmit information to others (Bro-Jørgensen, 2009). For example, in the strut displays of the greater sage grouse (*Centrocercus europhasianus*) males combine wing and tail movements with popping vocalizations to attract females (Wiley, 1973); the male wolf spider *Schizocosa stridulans* uses its abdomen and pedipalp simultaneously to produce different components of its complex sexual display (Elias, 2006); and guppies (*Poecilia reticulata*) show complex colour patches based on carotenoids, pteridines and melanins (Houde, 1997) as well as predator 'boldness', which is the individual willingness to risk approaching predators to 'inspect' them (Dugatkin & Godin, 1992; Wilson *et al.*, 1994; Godin & Davies, 1995).

In the European barn swallow *Hirundo rustica rustica*, several ornamental traits potentially used for sexual signalling purposes have been studied. Barn swallow males preferred as social and extra-pair mates have been found to have longer tail streamers (Møller, 1988, 1994; Saino *et al*., 1997), more symmetric outermost tail feathers (Møller, 1992, 1993b, 1994), larger white spots in the tail (Kose, 1999, Kose & Møller, 1999), a higher song rate (Møller *et al*., 1998), and a more intense red facial plumage (Ninni *et al*., 2004).

Amongst them, the one that has attracted most attention from researchers is an "expensive" signal, the elongation of tail streamers. Thus, males with longer tail streamers have been found to arrive earlier from wintering grounds in Africa (Møller, 1994), mate more often (Møller, 1994) and get more extra-pair copulations (Møller, 1988), have more broods (Møller, 1988, 1990, 1994), their females provide more parental care (Møller, 1991; de Lope & Møller, 1993), start breeding earlier and, overall, have a higher reproductive success (Møller, 1988, 1994). Furthermore, patterns of positive assortative mating by tail streamer length have been found (Møller, 1993b), suggesting a process of mutual sexual selection, and highlighting the quality-indicating value of such ornamental traits for European barn swallows. Also, some "cheaper" signals have been studied to figure out their relationship with tail length and with each other, and to see if they are having an effect in reproductive success. Perrier (2002), studied the dorsal plumage structural coloration, but he only found a weak and nonsignificant correlation with tail length and asymmetry and red facial colouration, as well as concluding that it was not condition dependent. Ninni *et al*. (2004) found a covariation between intense red facial plumage and the arrival date of barn swallows to breeding grounds, although the results were inconsistent: on first year of the study, early arriving animals had a duller red facial plumage than late arriving, whereas in second year the observed trend was just the opposite. Kose *et al.* (1999) and Kose & Møller (1999) found a positive correlation between white spots on tail feathers and tail length. The size of the white spots was also related to an earlier start of breeding.

In the North American barn swallow, a different subspecies (*Hirundo rustica erythrogaster*), however, although some evidence was found that females mated to males with experimentally lengthened tails bred earlier (Smith *et al.*, 1991; Smith & Montgomerie, 1991) and that streamer length predicted fertilization success (although effect was confounded by age; Kleven *et al.*, 2006), no significantly greater social or extrapair reproductive success was found for swallows with longer tail streamers (Smith *et al.*, 1991; Smith & Montgomerie, 1991). Safran & McGraw (2004) did not observe any advantages for individuals with larger or/and more symmetrical tails. However, ventral plumage coloration was found to predict patterns of assortative mating and reproductive success, two characteristic features of sexually selected traits (Safran & McGraw, 2004). In a series of colour manipulations carried out in North American barn swallow males, Safran *et al.* (2005) found an increased social paternity for males with enhanced ventral plumage colour.

Thus, two barn swallow subspecies, which have diverged relatively recently, approximately 100,000 years ago (Zink *et al.*, 2006; Dor *et al.*, 2010) use very different ornamental characters as the main quality indicators in inter-sexual signalling. This may be due to adaptation to different environments. A possible explanation for this variability in the main sexual signal used during mate choice could be that North American barn swallow males may need to take part in the egg incubation due to their habit of nesting on exposed sites. In these exposed sites, it may be more difficult to keep eggs at a proper temperature (Smith & Montgomerie, 1992) than in the case of the European barn swallows, which may prefer to nest in buildings in close association with humans, especially cowsheds (Møller, 1983), where temperature is typically warmer (Turner, 2006). For this reason, tail streamers in the North American barn swallow males may be more subject to breakage, and alternative mating systems and opportunities for sexual selection may have evolved as a consequence (Smith & Montgomerie, 1992).

In spite of the costs associated with production and reception of signals, animals often engage in multiple signalling systems. In a recent review, Bro-Jørgensen (2010), argues that variation in selection pressures derived from fluctuations in the environment (ecological conditions as well as social context) could be the factor which leads to the appearance and maintenance of multiple signals, both redundant and non-redundant, in animal systems. In the case of the barn swallow, this diversification of the sexual signals used to display individual quality could explain the fact that so many different ornamental traits have been found to have an effect in the reproductive success. Likewise, having multiple signals available with different production and maintenance costs (and thus differential information content) may explain why we still observe such a great variability in tail streamer length range (85-146 mm; Turner, 2006) in spite of this character being subjected to intense sexual selection by females. Actually, throat and ventral colouration might be more important ornaments in the European barn swallow than previously thought, and predict patterns of assortative mating and reproductive success, at least to a certain extent and in tune with the patterns predicted by tail streamer length.

Indeed, males with long tails take less time to acquire a mate. Furthermore, males which acquire a mate earlier will have a higher reproductive success than those pairing later in the season, due to decreasing probabilities of recruitment for nestlings and of producing a second clutch as the breeding season progresses (Møller, 1990b, 1994). Only males arriving earlier to the breeding grounds may be able to gain a reproductive success high enough as to balance the cost of an "expensive" signal such as elongated tail streamers. Thus, we hypothesize that males arriving later to the breeding grounds may be selected to decrease the investment in tail streamer elongation in favour of "cheaper" ornaments in order to tradeoff the amount of information signalled to mates with its costs. Also, we hypothesize that males with "cheaper" sexual signals may be selected to develop some sort of "compensation mechanism", such as a greater willingness to defend a breeding site associated with a higher quality foraging area, to outweigh their lower signalling intensity.

In this study, we investigate the effect, on the date of the first egg laid and on the quality of the foraging areas associated with breeding sites, of physical condition and several feather ornaments potentially used with sexual signalling purposes in the European barn swallow *Hirundo rustica rustica*: one more "expensive", the extremely elongated tail streamers, main quality indicator used by this subspecies, and another two "cheaper", throat and ventral plumage colouration, major quality indicators in the North American subspecies. Providing the date of the first egg laid is correlated with the date of arrival of the barn swallows to the breeding grounds (Turner, 2006), we predict that earlier-breeding males (and females, if they mate assortatively), will preferently have longer tail streamers, whereas later-breeding ones will show a higher investment in pigment-based plumage markings. Likewise, we predict a better physical condition for earlier-breeding barn swallows. Finally, in accordance with the previously explained hypothesis, we predict that breeding sites related to foraging areas with higher large insect abundance, a measure of habitat quality, will be preferently occupied by male (and female, if they mate assortatively) barn swallows with more elaborate (darker) pigment-based plumage displays.

Materials and Methods

Barn swallows are small- to medium-sized, socially monogamous semicolonial passerines. They are specialized on aerial-feeding, preying on insects caught on the wing, and they belong to the family of swallows and martins Hirundinidae (Møller, 1994; Turner, 2006).

Field work was carried out during March-September 2009 and 2010 in the surroundings of the University of Exeter Cornwall Campus, UK, in open farmland with scattered crop fields, pastures and hedgerows, where barn swallows breed in farms either solitarily or in colonies usually of up to about 10 pairs. Swallows were caught either during the day, using mist nets, or at night, using sweep nets, while they were roosting in their breeding sites. 112 adult barn swallows were caught across both years: 67 adults were caught in 2009 (33 males and 34 females) and 45 in 2010 (22 males and 23 females). Once caught, they were sexed according to the presence (females) or absence (males) of a brood patch in the belly area, ringed, several morphometric measurements taken (tail streamer length, tarsus and wing length), weighted and feather samples collected for subsequent objective colour assessment in the lab. Also, insect samples were collected in the barn swallows' foraging areas using Malaise traps for subsequent calculation of the amount of prey available in different breeding sites throughout the breeding season, and several weather variables (temperature, humidity and wind speed) measured in those areas. Nests were individually monitored every day or every other day throughout the breeding season to find out the date of the first egg laid, as well as the clutch size and the total number of chicks hatched and fledged.

Objective colour assessment

Colour was quantified using a USB2000 spectrometer (Ocean Optics, Dunedin, Florida), and a xenon flash lamp (Ocean Optics). We used a WS-1 SS Diffuse Reflectance Standard, a diffuse white plastic >98% reflective from 250-1500 nm, as the white reference (100% reflectance), and a piece of black velvet as the dark standard (0% reflectance) to correct for the noise when no light is reaching the sensor. At the far end of the reflection probe/light source, a non-reflective black pointer cut in a 45 degree angle was placed to avoid mismeasurement derived from the light reflected by the plumage reaching the sensor (Andersson and Prager, 2006; Pike *et al.*, 2011). Using the spectra acquisition software package OOIBase (Ocean Optics), we measured the reflectance of feathers collected from four body regions: the throat, breast, belly and vent of each bird. The pointer was applied to plumage patches "created" mounting the feathers on a flat surface in a way that simulates the original plumage structure, following a method that has been widely used in bird colouration studies (e.g. Cuthill *et al.*, 1999; Keyser & Hill, 2000; Perrier *et al.*, 2002; Safran & McGraw, 2004; Komdeur *et al.*, 2005; McGraw *et al.*, 2005; Quesada & Senar, 2006; Safran, 2007; Vaquero-Alba, Evans & Dall, unpublished data). Feathers were mounted on a piece of black velvet to avoid background noise.

As we are interested in the signalling strength of ventral colouration as a sexual indicator, we used the brightness, which is defined as the summed mean reflectance across the entire spectral range ($R_{300-700}$; Montgomerie, 2006), and is the best predictor of melanin content in barn swallows' feathers, with lower values, i.e. darker colours, indicating a higher total content of melanin (McGraw e*t al*., 2005; Galván and Møller, 2009).

Insect collection

Insect collection was carried out in Malaise traps, large and tent-like structures used for trapping flying insects, mainly diptera and hymenoptera. After hitting the central wall, insects tend to fly upwards and are then funnelled into a collecting vessel containing ethanol attached to the highest point.

Separated land patches or “fields” within an area of approximately 300 m around the nesting sites were located using aerial images of the sampling areas. Previous studies in English populations have found a greater number of foraging barn swallows within such ‘home ranges’ (Evans, 2001). After randomly assigning traps to these land patches, we placed them next to hedgerows, which are a good source of insects (Evans *et al*., 2003; Turner, 2006), and left them for approximately 3 or 4 hours. We sampled all the 14 sites four times during 2009 and twice during 2010, and spaced the sampling dates as evenly as possible across the breeding seasons. Between two and four sites were sampled every day during each sampling attempt, so that each of the attempts for the whole set of sites was carried out in up to four days. Two samples were taken at the same time in different locations in every site, as some of them were lost due to unstable ground, strong wind, curious cattle or other reasons.

Once collected, insects were counted, measured to the nearest millimetre, classified according to their size in small (<1-3mm), medium (4-7mm) and large (8->10mm) categories, abundance per hour calculated and quantities along the breeding season averaged for each size category within each site. Larger insects are the most energetically efficient ones for barn swallows (Turner, 2006), so we used this variable as an estimator of the habitat quality within the different breeding sites.

Statistical analyses

A principal component analysis (PCA) was conducted on the weather variables measured in the foraging areas (temperature, humidity and wind speed), and first principal component (PC1) was used in subsequent analyses. Principal component analysis and extraction of component loadings were conducted using the *principal* function of *psych* package (Revelle, 2011) in R (Crawley, 2007; R Development Core Team, 2010).

To test the relationship between the physical condition and the intensity of expression of the plumage traits between males and females within the pairs (i.e. assortative mating), we implemented several GLM models, in which “male ornament” or “male condition” was our dependent variable, whereas “female ornament” or “female condition” was the covariate and “year” the independent factor. By testing the effect of the interaction between year and female ornament/condition on male ornament/condition, we could control for the possible influence of yearly variations on assortative mating patterns (Quesada & Senar, 2009).

We analysed the variables influencing the selection of breeding sites by barn swallows according to the habitat quality of the foraging areas within them, using generalized linear mixed effects model (GLMM; details explained below) implemented with the *lmer* function of *lme4* package (Bates & Sarkar, 2007) of R 2.13.0 (Crawley, 2007; R Development Core Team, 2010). Using GLMMs, it is possible to analyse the influences of both random and fixed effects on response variables (Calabuig *et al.*, 2010).

Factors affecting the abundance of large insects were analysed using a Gaussian error structure and identity link function. The throat brightness, ventral brightness -calculated as the average of belly, breast and vent brightness-, tail length and condition -calculated as the ratio of the weight to the tarsus length, a good estimator of avian body size (Senar & Pascual, 1997) - were included as covariates, and sex was included as a fixed factor, whereas year of sampling was included as a random factor to control for its potential influence on large insect abundance. As habitat quality was calculated for each of the breeding sites, where there were several individuals, we calculated the coefficients of variation of throat and ventral brightness, tail length and condition for all the individuals of the same sex present in each site on each year. The coefficient of variation is defined as the ratio of the standard deviation of several measurements to their arithmetic mean (□= $s / \bar{x}$; Hendricks & Robey, 1936).

GLMMs were also used to analyse the effects of level of expression of sexual characters on the condition and reproductive performance of breeding pairs. Laying dates were transformed to Julian dates and analysed using a normal error structure and an identity link function, and so was condition. In all these analyses we included throat and ventral brightness, tail length and large insect abundance as covariates, to evaluate their effect in individual condition and reproductive performance. Weather was also included as a covariate to control for its potential confounding effects. As temperature was correlated with humidity (Pearson's correlation test, $r = -0.77$, $t_{110} = -12.5621$, $p < 2.2e-16$) and in order to reduce the number of variables accounting for weather conditions, we included all the weather variables measured (temperature, humidity and windspeed) in a principal component analysis (PCA) and actually included the first component (PC1) in the model. Sex was included as a fixed factor, and site, nest ID and year were included as random effects to control for possible differences in individual condition and breeding performance parameters between nest locations and years.

GLMMs were initially fitted with all explanatory terms included in them, including first-order interactions in all the models and second-order interactions in which several sexual ornaments (tail length, ventral or throat brightness) were involved for the insect abundance analysis. We chose our minimal adequate models after a stepwise deletion process of the least significant effects ($p>0.05$), starting with the highest-order interactions, providing the simplification did not significantly reduce the explanatory power of the model (Crawley, 2002; Schuett & Dall, 2010). Hypotheses were tested using chi-square ($\chi2$) statistics. The residuals from the models were checked for normality and homocedasticity.

Results

We found a significant and positive covariation between male and female physical condition ($F_{1,51}=6.477$, $p=0.014$; Table 1), and also between male and female ventral plumage brightness ($F_{1,43}=24.774$, $p<0.0001$; Table 1), in our study population. There was no significant covariation between male and female tail streamer length ($F_{1,52}=2.1063$, $p=0.1527$; Table 1) or between male and female throat plumage brightness ($F_{1,43}=1.8024$, $p=0.1865$; Table 1). None of these relationships was confounded by year effect.

The first principal component (PC1) of the principal component analysis (PCA) conducted for weather variables (temperature, humidity and windspeed) measured in barn swallows' foraging areas, explained more than a 71% of the overall amount of variance (Table 2).

The GLMMs showed a significant 3-way interaction between condition, ventral brightness and throat brightness on large insect abundance around breeding sites χ^2_1=9.9225, p=0.002; Table 3).

Breeding sites surrounded by areas with highest large insect abundance were occupied by individuals with both darker throat plumage patches and darker ventral plumage patches. Barn swallows with either darker throat plumage or darker ventral plumage bred in sites associated with foraging areas with a low large insect abundance, whereas those with brighter throat and ventral patches foraged in sites with a slightly higher large insect abundance (Figure 1).

Barn swallows with brighter throat plumage and in better physical condition bred in sites next to foraging areas richer in large insects. Those swallows with darker throat plumage and in poorer physical condition also occupied sites surrounded by foraging areas with high large insect abundance, but slightly lower in this case. Darker-throated individuals in better physical condition and brighter-throated ones in poorer condition bred in sites surrounded by areas with much lower large insect abundance (Figure 2).

For the effect of the interaction between ventral brightness and condition on large insect abundance, the trends were similar to the above interaction, with greater amounts of large insects found for areas occupied by darker swallows in poorer condition, and less but still high abundance in areas where less dark individuals in better condition foraged (Figure 3).

Our analyses also showed a significant 2-way interaction between tail length and ventral brightness on large insect abundance around breeding sites (χ^2_1=8.306, p=0.004; Table 3, Figure 4). Individuals with brighter ventral areas bred in sites associated to foraging areas with greater amount of large insects, and this effect was more pronounced for swallows with shorter tail streamers. For swallows with darker ventral areas, abundance of large insects in areas around breeding sites was very low, and slightly higher for longer-tailed swallows (Figure 4).

Finally for the habitat quality analysis, we found a 2-way interaction between throat brightness and sex on large insect abundance (χ^2_1=6.468, p=0.011; Table 1). The graphical plot revealed that males with darker throat plumage patches bred in sites surrounded by foraging areas with greater large insect abundance (Figure 5), whereas there was no clear pattern for females.

When analysing the date of the first egg laid as the response variable, we found a significant effect of body condition on laying date (χ^2_1= 8.359, p=0.004; Table 3). Individuals in better physical condition started their first breeding attempt significantly earlier (Figure 6). We also found a weak but significant 2-way interaction between throat brightness and sex on the laying date of the first egg (χ^2_1=5.001, p=0.025; Table 3). Males with darker throat colouration patches were mated to females laying their first eggs earlier (Figure 7). No such effect was observed for female throat colouration.

Discussion

As we predicted, barn swallows with further elaboration of “cheaper” sexual ornaments seem to actively seek higher habitat quality breeding sites: darker-throated males occupied better quality breeding sites according to large insect abundance in the surrounding areas. Males and females with simultaneously darker throat and ventral plumage patches also bred in sites associated with higher quality foraging areas. So did individuals with either poorer body condition and darker throat or ventral colouration, or better condition and less dark plumage. Thus, barn swallows investing in “cheaper” ornaments, but also those with some kind of “weakness” in their sexual displaying performance or physical condition, seem to be selected to actively look for breeding sites close to higher-quality habitat patches. We observed patterns of positive assortative mating by ventral brightness and body condition, which highlight the condition-dependence and quality-indicating value of ventral brightness for European barn swallows (Safran & McGraw, 2004) and suggest the existence of mutual mate choice (Bitton, 2008). Females mated to darker-throated males laid significantly sooner, and so did females in better condition and/or mated to males in better physical condition. The results of our study suggest that throat and ventral plumage colouration function as quality-indicating traits in the European barn swallow, and suggest a "compensation mechanism" through habitat quality for individuals with impaired displaying ability. It's the first time, to the best of our knowledge, that evidence for the quality-indicating value of pigment-based ornaments is found for the European bar swallow *Hirundo rustica rustica*.

We did not detect assortative mating patterns by tail streamer length in our population. The assortative mating pattern detected was more in accordance with the results of studies conducted in the North American barn swallow *Hirundo rustica erythrogaster*, where ventral colouration is the most important ornament used for sexual signalling purposes and is closely related to reproductive success (Safran & McGraw, 2004).

In other studies conducted on territorial species, territory attractiveness has been positively correlated with several reproductive success indicators (Hasselquist, 1998), and female choice was determined by an interaction of male and territory quality (Yasukawa, 1981). Even if barn swallows are not strictly territorial, males defend a breeding site of a few square metres (Turner, 2006). Quality of the foraging area surrounding it may be an important factor when selecting and defending a given breeding site. In our population, nesting sites surrounded by areas with a higher large insect abundance are preferently occupied by males with darker throats. Our results suggest that males with a higher investment in sexual cues related to colouration (like throat brightness), rather than tail streamers, may invest more in defending nesting sites associated with better foraging areas, as we predicted. If males and females assess the quality of nesting sites and foraging areas associated to them in a similar way, females may be using habitat quality as an indirect cue to select higher quality males. Alternatively, they may be selecting males with a higher investment in throat plumage colouration, in order to grant access to higher habitat quality sites.

In the tree swallow, a territorial species related to the barn swallow, males with territories were in better condition than floaters (Lozano, 1994). In our study, however, individuals with some sort of "weakness", like poorer physical condition or brighter throat or ventral colouration, seemed to occupy breeding sites associated with a high quality foraging area, perhaps compensating for the lack of reproductive success derived from the aforementioned weakness. Swallows with both darker throat or ventral colouration and a good physical condition tended to occupy sites with a lower abundance of large insects. As these individuals were already indicating at a considerably high level, at least for colouration traits, they might not have any constraint to engage in a "compensation mechanism".

Likewise, there were fewer large insects in sites occupied by brighter individuals in a poorer physical condition. Several possibilities have been proposed to explain the coexistence of multiple ornaments (Bro-Jørgensen, 2009; Freeman-Gallant, 2010): the "multiple messages" hypothesis (van Doorn & Weissing, 2004) suggests that all the signals might be honest quality indicators of male quality if each of them conveys information about different attributes or targets different receivers. Thus, our results might suggest that plumage colouration in barn swallows is involved in agonistic male-male interactions rather than in mate choice: if darker throat and ventral plumage reflect a greater ability to defend a breeding site, we might expect to see individuals with darker plumage patches occupying sites associated with foraging areas richer in large insects, as we did. Also, individuals with brighter patches but in a better physical condition have much to win from getting access to a higher quality foraging area. It may be a way to compensate their low level of display to the females at a low cost (low ornament production/maintenance costs), saving themselves for future reproductive attempts . Dark individuals in a good condition may be able to naturally attract more females, so they might not have so much to win from escalating too much in an agonistic interaction for a breeding site. Bright individuals in poorer physical condition might suffer from an impaired ability to win agonistic interactions for breeding sites.

Although tail length alone was not found to have any effect on the quality of the foraging sites selected by barn swallows in our study, in interaction with ventral brightness it influenced the large insect abundance of the foraging areas associated with the breeding sites occupied by barn swallows. Individuals with brighter ventral patches selected breeding sites associated with foraging areas richer in large insect abundance, an effect that was more pronounced for individuals with shorter tail streamers. Tail length did not seem to have such a strong effect on the quality of the breeding site foraging areas occupied by barn swallows, and only for lower values of ventral brightness could we see a trend in shorter tailed individuals towards selecting areas with a greater abundance of large insects. So, individuals with a lower intensity in the expression of two different ornaments selected breeding sites associated with better quality foraging areas. This behaviour may compensate for the lack of attractiveness of those ornaments with a lower signalling strength. For individuals with longer tail streamers, however, there was a less intense effect of ventral brightness on the quality of preferred areas. As male barn swallows with longer tail streamers have been found to have a higher overall reproductive success (Møller, 1988, 1994), they may not be selected to invest so much in compensating for the lack of intensity in the expression of ventral plumage colouration as the ones with shorter tail streamers.

Surprisingly, in interaction with tail length, the influence of ventral brightness in the quality of the selected foraging areas has the opposite effect to the one it has when it interacts with throat brightness. This may be because feather-size related and colouration-related ornaments impose different constraints in terms of production and/or maintenance costs and information content. Thus, throat and ventral colouration, when considered together, seemed to have a synergic effect on the quality of the foraging sites associated with the breeding sites selected by their bearers. When physical condition or tail length, a more "expensive" signal of individual viability, were considered, the effect of each of the "cheaper" plumage colouration ornaments in breeding site preferences varied, in order to satisfy the needs imposed by different combinations of costs and benefits.

Females mated to males with lower values of throat brightness (i.e. darker throat patches) tended to have an earlier laying date for their first egg. Likewise, physical condition also had an effect on laying date, so that individuals in better condition bred significantly earlier. As barn swallows in the population we studied mated assortatively by body condition, we did not detect any influence of sex in the relationship between laying date and condition, as we did for covariation between throat brightness and laying date (there was no assortative mating by throat brightness in our population). These results are in concordance with previous studies. Ninni *et al.* (2004) found evidence of condition-dependence in the date of the first egg laid by barn swallows in a Spanish population, as well as an effect of the colour of the red facial feathers on the laying date, although results were not consistent for the two years studied. However, contrary to several studies that have found a strong negative relationship between date of arrival to the breeding grounds and laying date in barn swallows, and the length of the outermost tail feathers, i.e. the tail streamers (e.g. Møller, 1994, Ninni *et al.*, 2004, Teplitsky *et al.*, 2011), we did not observe any effect of tail streamer length on the laying date of the first egg, nor an assortative pairing pattern by tail length between males and females. Throat brightness, rather than tail streamer length, seemed to be the most important plumage ornament related to laying date, an indicator of reproductive success.

To sum up, throat and ventral plumage colouration seem to play an important role in the sexual signalling system of the European barn swallow. The results of our study suggest that they are part of a multicomponent signalling system which may have evolved as a response to dynamic selection pressures caused by fluctuations in ecological and social conditions (e.g. changes in farming activity, climate change and other factors may affect the signal expression in barn swallows). Future studies should be carried out to find out whether relative importance of each of the sexual signals taking part in multiple-signalling systems in the barn swallow might be changing as part of a dynamic process, as our results suggest. We also found evidence for a "compensation mechanism" by which individuals with lower displaying rates in one or more sexually selected traits should try to compensate their lack of reproductive fitness by defending higher quality breeding sites.

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Table 1: GLM models within mated pairs, relating the intensity of expression of male ornaments (tail length, throat brightness and ventral brightness) and physical condition to those of their female partner (i.e. assortative mating), controlling for the effect of the year

Male ornament	Year:Female ornament		Year		Female ornament	
	Test	p	Test	p	Test	p
Colouration						
Throat brightness	$F_{1,41}$=0.150	0.701	$F_{1,42}$=0.0004	0.984	$F_{1,43}$=1.802	0.187
Ventral brightness	$F_{1,41}$=0.230	0.634	$F_{1,42}$=0.017	0.899	$F_{1,43}$=24.774	<0.0001 ***
Tail length	$F_{1,51}$=3.249	0.078	$F_{1,51}$=1.221	0.274	$F_{1,52}$=2.106	0.153

Male condition	Year:Female condition		Year		Female condition	
	Test	P	Test	P	Test	p
Condition	$F_{1,49}$=0.783	0.380	$F_{1,50}$=1.119	0.295	$F_{1,51}$=6.477	0.014*

Table 2: Component loadings (unrotated) of first principal component (PC1) as quantified by principal component analysis

Variable	Mean	SD	Principal Component PC1
Temperature	24.596	1.797	**-0.90**
Humidity	47.380	6.961	**0.92**
Wind speed	8.688	3.795	**0.70**
Eigenvalue			2.137
% variance explained			71

Loadings over ±0.6 are marked in bold. *N* = 112

Table 3 Summary of minimal adequate models

Response variable	N	ΔAIC	Explanatory variable	χ^2(df)	p
Large insect abundance[a]	31	6.03	Tail length	13.94 (1)	0.0002
			Ventral bright	8.733 (1)	0.003
			Throat bright	9.856 (1)	0.002
			Condition	11.005 (1)	0.0009
			Sex (male)	3.051 (1)	0.081
			Ventral bright:throat bright	14.565 (1)	0.0001
			Ventral bright:condition	14.016 (1)	0.0002
			Throat bright:condition	6.926 (1)	0.008
			Tail length:ventral bright	8.306 (1)	0.004
			Throat bright:sex (male)	6.468 (1)	0.011
			Ventral bright: throat bright:condition	9.922 (1)	0.002
Laying date[b]	83	18.6	Sex (male)	0.286 (1)	0.593
			Throat bright	92.415 (1)	<0.0001
			Condition	8.3586 (1)	0.004
			Throat bright:sex (male)	5.0198 (1)	0.025

ΔAIC difference between AIC of maximal and minimal model, *condition* physical condition, covariate, *large insects* large insect abundance, covariate, *nest:site* nest location within each site, factor with 35 levels, *sex* factor with 2 levels, *site* geographic location of nesting site, factor with 12 levels, *tail length* tail streamer length, covariate, *throat bright* throat brightness, covariate, *ventral bright* ventral brightness, covariate, *weather* weather measurement, covariate, *year* year of study, factor with 2 levels

[a] Fixed effects and covariates included in maximal models: tail length, ventral bright, throat bright, condition, sex, tail length:ventral bright, tail length:throat bright, ventral bright:throat bright, tail length:condition, ventral bright:condition, throat bright:condition, tail length:sex, ventral bright:sex, throat bright:sex, condition:sex, tail length:ventral bright:throat bright, tail length:ventral bright:condition, tail length:throat bright:condition, ventral bright:throat bright:condition. Random effects: year.

[b] Fixed effects and covariates included in maximal models: sex, ventral bright, throat bright, tail length, condition, large insects, weather, ventral bright:sex, ventral bright:large insects, ventral bright:weather, ventral bright:tail length, ventral bright:throat bright, ventral bright:condition, throat bright:sex, throat bright:large insects, throat bright:weather, throat bright:tail length, throat bright:condition, tail length:sex, tail length:large insects, tail length:weather, tail length:condition, condition:weather, condition:large insects. Random effects: year, site/nest.

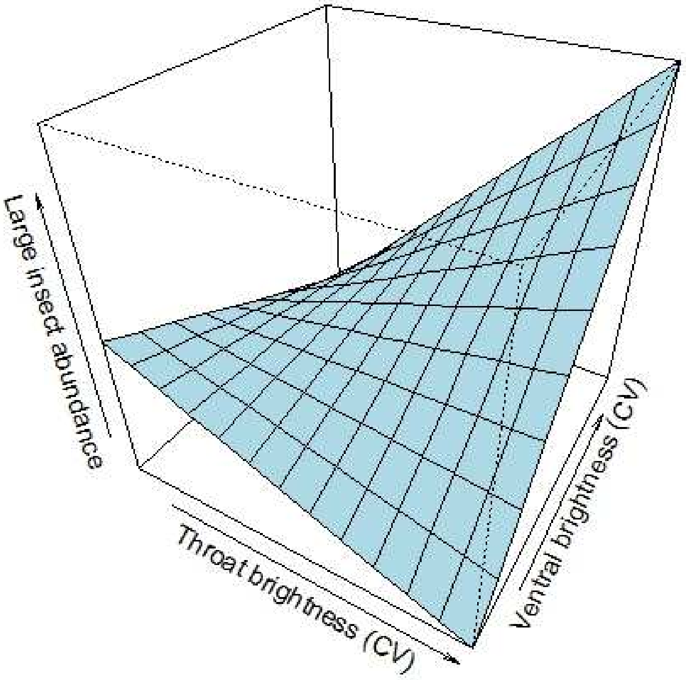

Figure 1: The effect of throat and ventral brightness on large insect abundance (CV=coefficients of variation)

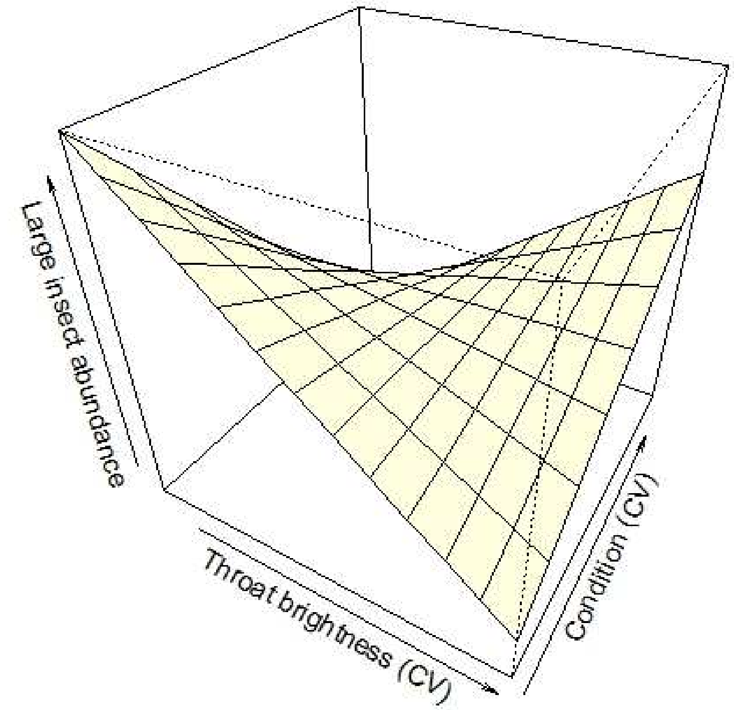

Figure 2: The effect of throat brightness and condition on large insect abundance (CV=coefficients of variation)

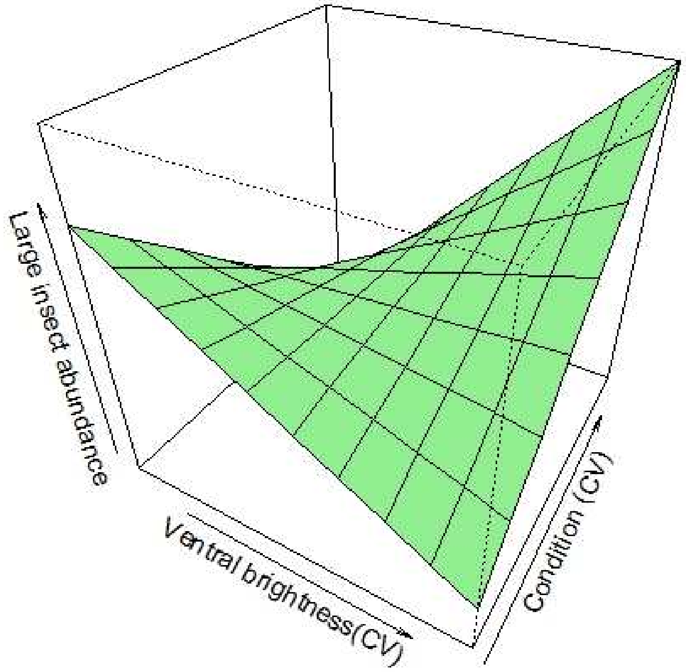

Figure 3: The effect of ventral brightness and condition on large insect abundance (CV=coefficients of variation).

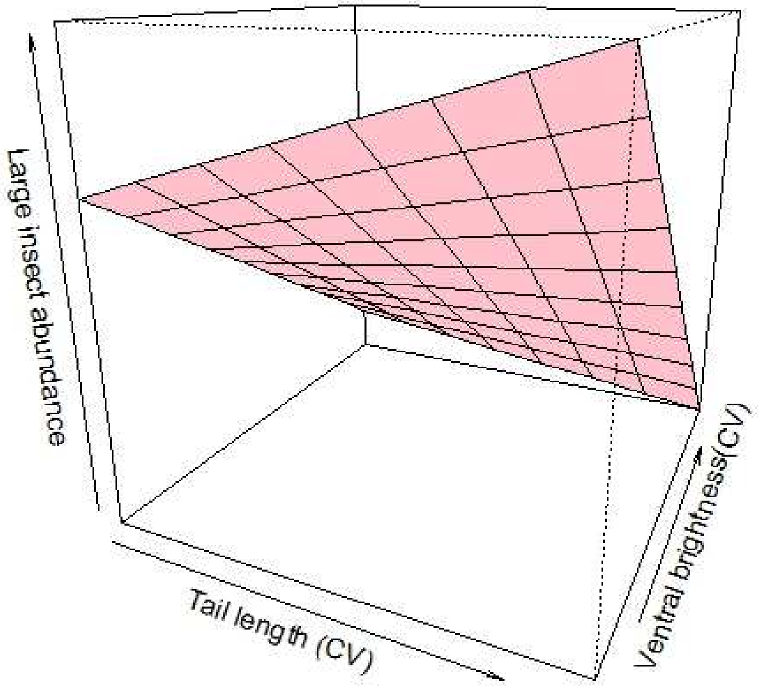

Figure 4: The effect of tail length and ventral brightness on large insect abundance (CV=coefficients of variation)

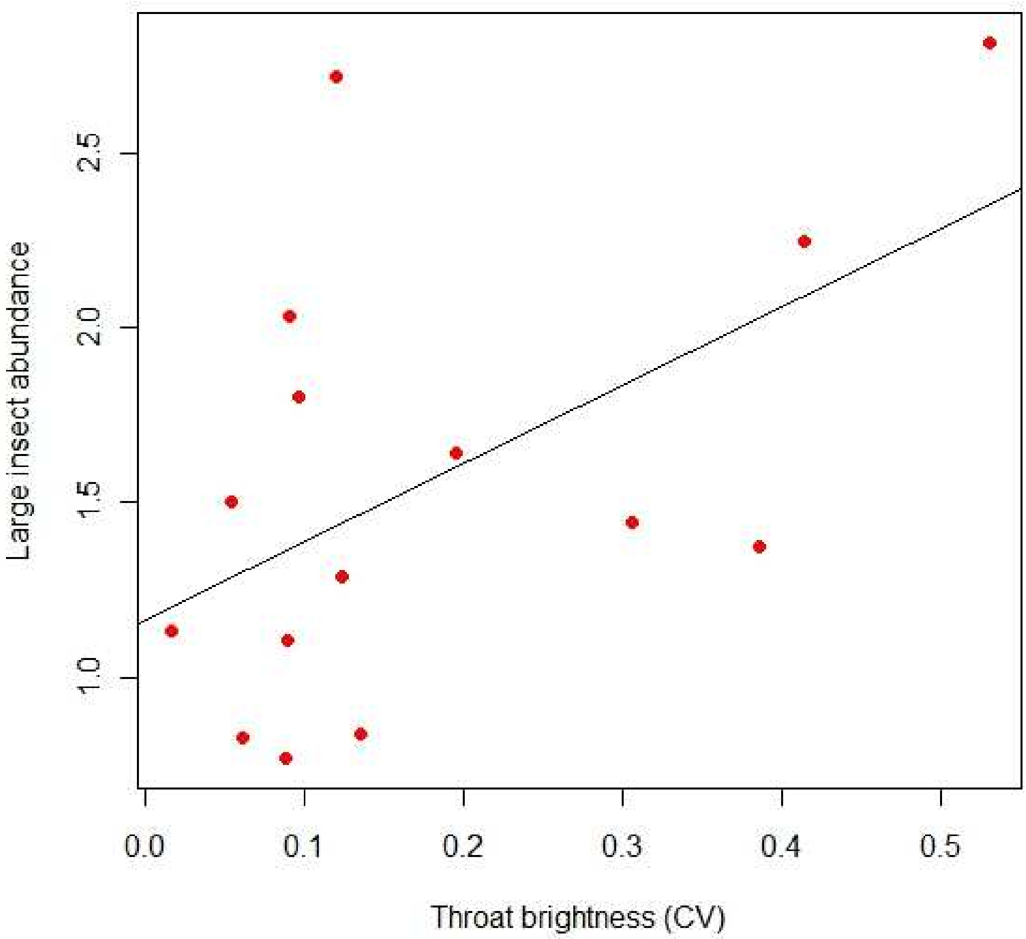

Figure 5: Effect of male throat brightness on large insect abundance (CV=coefficients of variation)

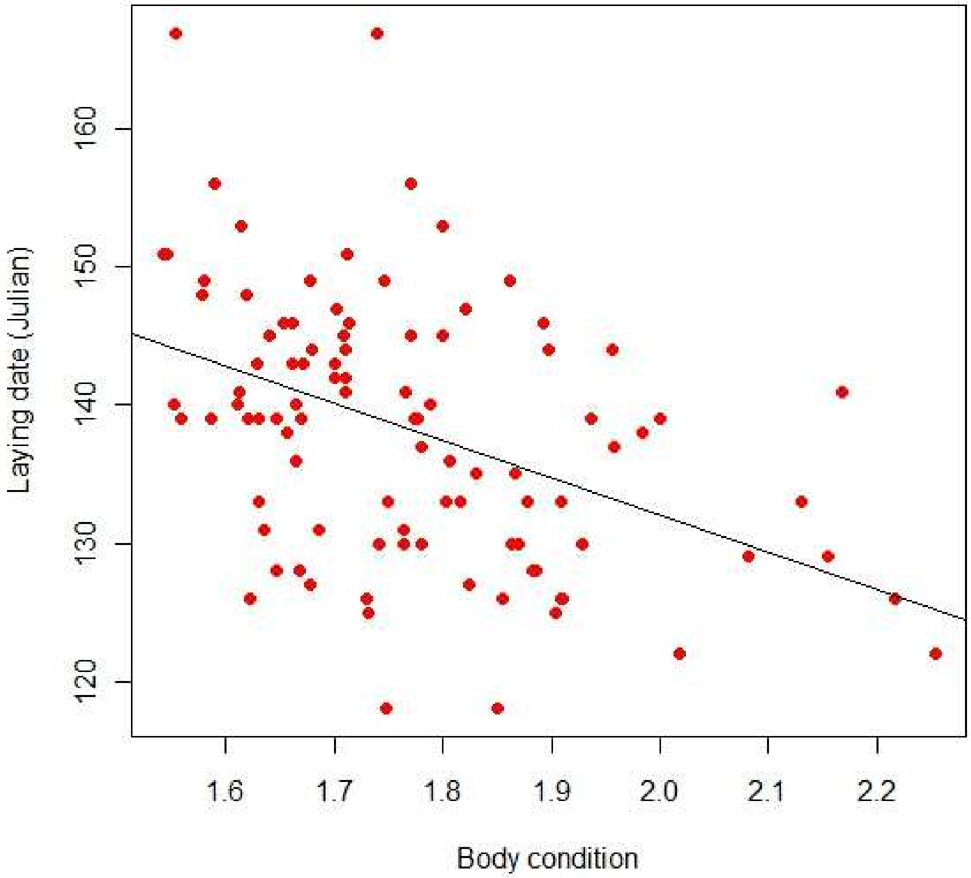

Figure 6: Effect of body condition on laying date

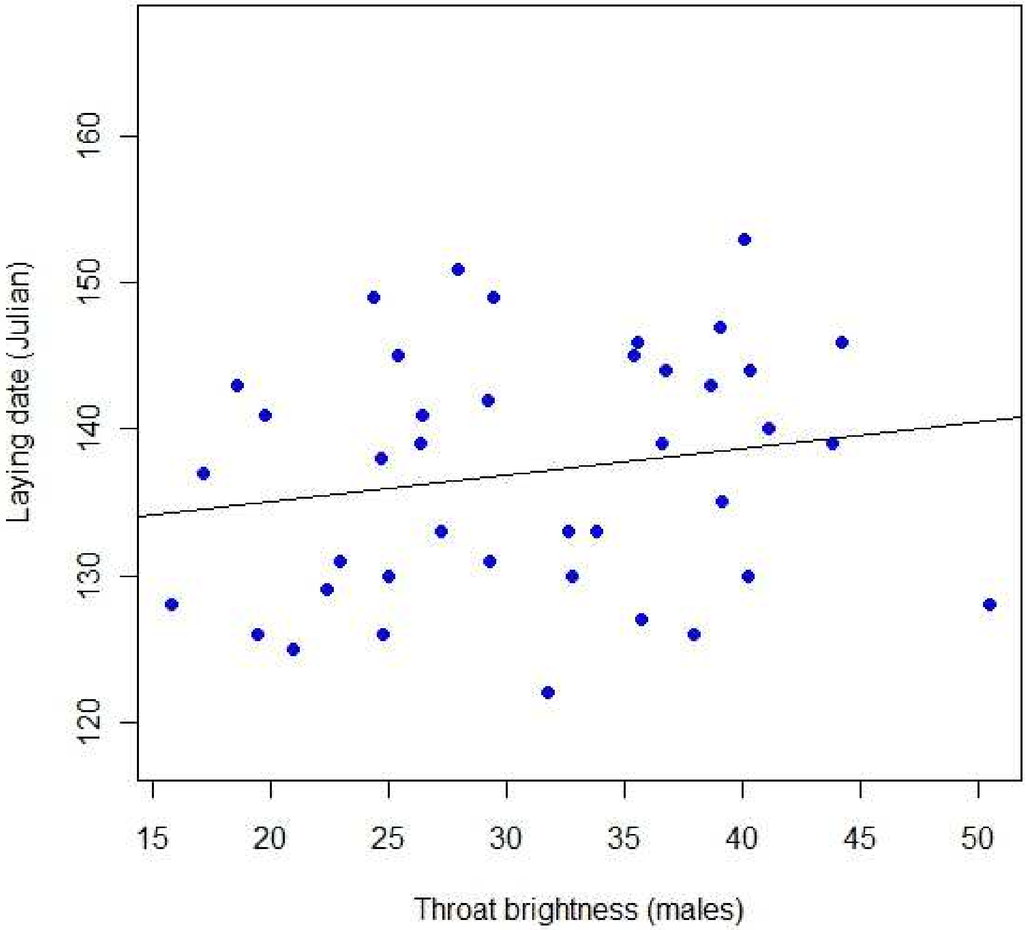

Figure 7: Effect of throat brightness of males on laying date

Chapter Four

Plumage colouration, as well as tail-streamer length, is a sexually selected trait in European barn swallows

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Abstract

Choosing elaborately ornamented individuals as sexual partners can provide animals with direct and indirect benefits. As a result, birds with more elaborate and colourful displays often enjoy a selective advantage, being preferred as mates. Animals often use more than one signal to transmit information to others. The main reason for the emergence and maintenance of multiple signals, both redundant and non-redundant, is variation in selection pressures derived from fluctuations in the environment. We studied, in the European barn swallow, the effects of several potentially sexually selected ornaments, along with plumage manipulations, on a series of measures of reproductive success. It is the first time, to the best of our knowledge, that throat and ventral colouration as well as tail streamer length, have been investigated from a multi-component signalling perspective in this subspecies. Most significantly, we found that having darker plumage patches, alone or in interaction with other factors, was related to higher levels of reproductive success by male swallows. Given the well known impact of tail streamer length variation on male swallow mating success, this suggests the existence of a multi-component signalling system for the European barn swallow and highlights the important contribution of colouration-related sexual signals to this system.

Introduction

Models of sexual selection suggest that choosing elaborately ornamented individuals as sexual partners can provide animals with direct and indirect benefits. Inter-individual differences in the degree of exaggeration of the ornaments transmit useful information about the benefits involved (Pomiankowski, 1987; Andersson, 1994; Garamsziegi *et al.*, 2006; Bro-Jørgensen *et al.*, 2007). Birds with more elaborate and colourful displays enjoy a selective advantage, as they are preferred as mates (Hill, 2006). Theoretical models also predict that these ornamental characters will be costly at evolutionary stability (Fisher, 1930; Zahavi, 1975; Iwasa *et al.*, 1991). This cost may be expressed at the moment of production and/or maintenance, and also continuously through increased risk of predation or parasitism (Magnhagen, 1991; Folstad & Karter, 1992). Costly signals are efficient quality-indicators, as the costs they inflict to their bearers make them likely to be general viability indicators, in accordance with the handicap principle (Zahavi, 1975). Sexual ornaments are quality-indicating signals kept honest (informative) by the handicap of their costs (Fitzpatrick, 2000).

Birds, as well as many other animals, often use more than one signal to transmit information to others (Bro-Jørgensen, 2009). For example, males of the greater sage grouse (*Centrocercus europhasianus*) combine wing and tail movements with popping vocalizations in their female-attracting strut displays (Wiley, 1973); and the male wolf spider (*Schizocosa stridulans*) produces different components of its complex sexual display by using its abdomen and pedipalp simultaneously (Elias, 2006). Animals often deploy multiple signalling systems, despite the costs associated with production and reception of signals. The main factor leading to the emergence and maintenance of such multiple signals, both redundant and non-redundant, is variation in selection pressures derived from fluctuations in the broader environment (including ecological conditions and/or social context; Bro-Jørgensen, 2009). Animals might be selected to utilize "cheaper" signals when the day-to-day costs incurred by the costlier signals have particularly severe fitness consequences. Likewise, they might minimise the elaboration of the costliest traits, such as tail elongation (Fitzpatrick, 2000) in such contexts. Therefore, birds may have evolved plumage that trades off the amount of information it signals to potential mates/rivals with its costs; in other words, such traits may be selected to optimise rather than maximise their information content.

Several ornamental traits have been studied in the European barn swallow *Hirundo rustica rustica* and argued to have a role in sexual signalling. For example, males preferred as social and/or extra-pair mates have longer and more symmetric tail streamers (Møller, 1988, 1992a, 1993, 1994; Saino *et al.*, 1997), larger white spots in the tail (Kose *et al.*, 1999, Kose & Møller, 1999) or more intense red facial plumage (Ninni *et al.*, 2004). Most of the studies of sexual selection in the European barn swallow have focused on variation in the elongated outermost tail streamers. Indeed, evidence is accumulating for a sexual function for these feathers over the last few decades (reviewed in Møller, 1994; Møller et al., 1998b). Although a mechanical and aerodynamic function of tail streamers in tight manoeuvres has been reported (Norberg, 1994), it is commonly agreed that they constitute a serious handicap in flight. Thus, sexual selection constitutes at least a partial explanation for exaggerated tail length in male barn swallows (Buchanan & Evans, 2000; Rowe et al., 2001, Møller, 1994). Males with longer tails mate more often, get more extra-pair copulations, have more broods and overall have higher reproductive success (Møller 1988, 1990, 1994). In addition, other types of signals have been studied to investigate their role in sexual selection and reproductive success, and their relationship with tail length. For example, Kose *et al.* (1999) and Kose & Møller (1999) studied white spots on tail feathers, finding a positive correlation between their size and tail length. The size of the spots was also related to an earlier start of breeding. However, Perrier *et al*. (2002) did not find a significant correlation between the dorsal plumage structural coloration, tail length, tail asymmetry and red facial colouration. Saino *et al*. (2003) did not find a reduction in singing activity or song complexity of males with artificially elongated tail feathers, suggesting that males are not displaying multiple signals at their maximum possible levels.

Nevertheless, few studies have been conducted in the European barn swallow to study the possible role of sexual selection on ventral plumage colouration (but see Ninni *et al*., 2004 and Galván & Møller, 2009 for studies on the red throat patch). Indeed, ventral plumage colouration predicts pairing patterns and reproductive success in the North American barn swallow subspecies *erythrogaster*. Safran *et al*. (2005) found increased social paternity rates (i.e. paternity from their social mates, as opposed to extra-pair paternity) in males with experimentally enhanced ventral plumage darkness. Although evidence suggests that this ventral plumage colouration is the main quality indicator in inter-sexual signalling for the North American subspecies, tail length still plays a role in sexual signalling: females mated to males with experimentally lengthened tails breed earlier (Smith & Montgomerie, 1991; Smith *et al*., 1991) and streamer length predicts fertilization success (although this effect is confounded by age; Kleven *et al*., 2006). If both ornamental characters: tail streamer elongation and ventral plumage colouration, were already used by barn swallows as quality indicators before the European and the North American subspecies diverged, both ornaments may play roles in sexual signalling in both subspecies at present. Variation in the relative importance of different signal types on the different continents may be due to adaptation to distinct local environmental conditions.

We hypothesize that ventral plumage colouration, as well as elongated tail streamers, function as quality indicators and determine reproductive success in the European barn swallow *Hirundo rustica rustica*, as part of a multi-component signalling system, in response to variation in local selection pressures. In the present study, we investigate the relationship between several common measures of reproductive success (clutch size, hatching success, physical condition of the chicks and food provisioning of chicks) and several feather ornaments potentially used for sexual signalling purposes. The ornaments considered are likely to differ in their production and maintenance costs, and therefore information contents: the elongated tail streamers (the main quality indicator used by this subspecies) are likely to be costly to both produce and maintain and thus have a high quality-indicating value, while the throat and ventral plumage colouration (the dominant quality indicators in the North American subspecies) should be costly to produce but less costly to maintain and therefore offer less information to prospective mates . We included female ornaments in the study because it is still not clear whether they are also subject to sexual selection or just nonadaptive, correlated effects of selection on males (Møller, 1993; Cuervo et al., 1996; Amundsen, 2000; Safran & McGraw, 2004; Kraaijeveld *et al*., 2007). Before the beginning of the second clutch, we also conducted a series of manipulations of male tail streamer length and ventral plumage darkness and measured their impacts on the fitness measures. If these ornaments are part of a multi-component signalling system that has evolved via variable selection (Bro-Jørgensen, 2009), and males are not displaying multiple signals at maximum possible levels (Saino et al., 2003), we predict naturally longer-tailed and darker-coloured individuals will show higher reproductive success . Furthermore, we predict increased reproductive performance for pairs whose male's ventral darkness was artificially enhanced, especially for those with shortened or unmodified tail streamers, and decreased performance for couples in which male tail length, the costliest ornament, was lengthened.

Materials and Methods

Barn swallows are small (approx. 20 g), semicolonial, socially monogamous passerines, feeding on insect prey caught on the wing, belonging to the family of swallows and martins Hirundinidae (Møller, 1994; Turner, 2006). They have a U-shaped tail with extremely elongated outermost feathers (called tail streamers), and they display patches of rust-colored plumage in the throat and ventral region, this trait being much more exaggerated in the North American subspecies (*Hirundo rustica erythrogaster*), but still present in the European subspecies (*H. rustica rustica*; Møller, 1992b, Safran & McGraw, 2004).

Field work was carried out in the surroundings of the University of Exeter Cornwall Campus, UK, during March-September 2009 and 2010. The study area was open farmland with scattered crop fields, pastures and hedgerows, where barn swallows breed in farms either solitarily or in colonies usually of up to about 10 pairs. 112 adult barn swallows were caught across both years: 67 adults in 2009 (33 males and 34 females) and 45 in 2010 (22 males and 23 females). Most of the swallows were caught during the day, using mist nets placed into the buildings where they nest. The remaining ones were caught at night, using sweep nets, while they were roosting in their breeding sites. They were then sexed according to the presence (females) or absence (males) of a brood patch in the belly area, and provided with a numbered aluminium band. Several morphometric measurements were taken: tail streamer and wing length, using a ruler, and tarsus length, using an electronic caliper. They were weighted, using a digital precision balance, and feather samples collected for subsequent objective colour assessment in the lab. Insect samples were collected in the barn swallow foraging areas using Malaise traps, for subsequent calculation of the amount of prey available in different breeding sites throughout the breeding season. Nests were individually monitored every day or every other day throughout the breeding season to record the size of the clutch and hatching success, calculated as the proportion of the eggs successfully hatched within one clutch. Within their first two weeks of life, chicks were weighted and their wing and tarsus length measured, using the same methodology described for adults. Physical condition of both adult barn swallows and chicks was calculated as the ratio of the weight to the tarsus length, a reliable estimator of avian body size (Senar & Pascual, 1997), and condition of the chicks within a nest was averaged for the statistical analyses.

In 2009, just after the end of the first successful breeding attempt and before the start of the second, 30 males were recaptured and their tail streamer length and ventral colouration brightness manipulated, whereas in 2010 males were not recaptured, and they were left unmanipulated. Tail manipulations were carried out as described by Møller and colleagues (reviewed in Møller, 1994), but elongating or reducing tail streamer length only by approximately 13 mm instead of 20 mm, to avoid interfering with the viability-selected part of the streamers (Buchanan & Evans, 2000; Rowe *et al.*, 2001). After recapture, for tail manipulations, males were randomly assigned to one of two tail streamer manipulation groups (elongation or reduction) or to a control group, where feathers were cut and reglued without length modification, and for colour manipulations, they were randomly assigned to a colour enhancement (darkening) group or to a control group, where plumage colouration was not modified. See Table 2 for a summary of the levels within the manipulation factor. The handling and manipulation of the adult barn swallows and chicks were made under ringing licenses from BTO and/or a license from Natural England.

Objective colour assessment

Plumage colour (before manipulation) was quantified using an Ocean Optics USB2000 spectrophotometer (range 250-800 nm) and a xenon flash lamp (Dunedin, FL). We used a "Spectralon" tablet (WS-1 SS Diffuse Reflectance Standard, Ocean Optics, Dunedin, FL), a diffuse white plastic >98% reflective from 250-1500 nm, as the white reference (100% reflectance), and a piece of black velvet as the dark standard (0% reflectance) to correct for the noise when no light is reaching the sensor. At the far end of the reflection probe/light source, we placed a non-reflective black pointer cut in a 45 degree angle to avoid mismeasurement derived from the light reflected by the plumage reaching the sensor (Andersson and Prager, 2006; Pike *et al.*, 2011). We measured the reflectance of feathers collected from four body regions: the throat, breast, belly and vent of each bird, using the spectra acquisition software package OOIBase (Ocean Optics). The pointer was placed on plumage patches "created" by mounting the feathers on a flat surface in a way that simulates the original plumage structure, following a method widely used in bird colouration studies (e.g. Cuthill *et al.*, 1999; Keyser & Hill, 2000; Perrier *et al.*, 2002; Safran & McGraw, 2004; Komdeur *et al.*, 2005; McGraw *et al.*, 2005; Quesada & Senar, 2006; Safran, 2007; Vaquero-Alba, Evans & Dall, unpublished data; **2**). Feathers were mounted on a piece of black velvet to avoid background noise.

We used the brightness, defined as the summed mean reflectance across the entire spectral range ($R_{300\text{–}700}$; Montgomerie, 2006), for our analyses, as we are interested in the signalling strength of ventral colouration as a sexual indicator. Brightness is the best predictor of melanin content in barn swallows' feathers, with lower values, i.e. darker colours, indicating a higher total content of melanin (McGraw e*t al*., 2005; Galván and Møller, 2009). We used two colour-related measures in our analyses: throat brightness and ventral brightness, calculated as the average of belly, breast and vent brightness.

Insect collection

Insects were collected in Malaise traps, which are large, tent-like structures used for trapping flying insects, mainly diptera and hymenoptera. When insects hit the central wall, they tend to fly upwards and are then funnelled into a collecting vessel containing ethanol, which is attached to the highest point.

Using aerial images of the sampling areas, separate land patches or "fields" within an area of approximately 300 m around the nesting sites were located. Previous studies in English populations have found a greater number of foraging barn swallows within such 'home ranges' (Evans, 2001). Traps were randomly assigned to these land patches, placed next to hedgerows, which are a good source of insects (Evans *et al*., 2003; Turner, 2006), and left for approximately 3 or 4 hours. All the 14 sites were sampled four times during 2009 and twice during 2010, spacing the sampling dates as evenly as possible across the breeding seasons. Each time, between two and four sites were sampled every day, so that each of the collection attempts for the whole set of sites was carried out in up to four days. In every site, two samples were taken at the same time in different locations, as some of them were lost due to unstable ground, strong wind, curious cattle or other reasons.

After collection, insects were counted, measured to the nearest millimetre, classified according to their size in small (<1-3mm), medium (4-7mm) and large (8->10mm) categories, abundance per hour calculated and quantities along the breeding season averaged for each size category within each site. As larger insects are the most energetically efficient ones for barn swallows (Turner, 2006), we used their abundance as an estimator of the habitat quality within the different breeding sites chosen by different pairs.

Statistical analyses

We analysed the variables influencing several indicators of reproductive success in the barn swallows, using generalized linear mixed effects models (GLMM; details explained below) implemented with the *lmer* function of *lme4* package (Bates & Sarkar, 2007) of R 2.13.0 (Crawley, 2007; R Development Core Team, 2010). Using GLMMs, it is possible to analyse the influences of both random and fixed effects on response variables (Calabuig *et al*., 2010). As plumage manipulations were carried out before the start of the second clutch in 2009, measurements of reproductive success were not comparable across both clutches. For that reason, we decided to analyse the 1st and 2nd clutches separately, instead of pooling data together.

Factors affecting the size of the clutch were analysed using a Poisson error structure and a log link function. The throat brightness, ventral brightness, tail length, physical condition and large insect abundance were included as covariates, and sex was included as a fixed factor. In the analyses corresponding to the second clutch, plumage modification was also included as a fixed factor. Factors affecting the hatching success and the physical condition of chicks were analysed using a Gaussian error structure and an identity link function. The same covariates and fixed factors as before were included in the analyses. Site identity, nest location and year were included as random effects to control for possible differences in reproductive investment and breeding performance parameters among sites, nest locations within sites and years. In the analyses of the factors affecting physical condition of chicks, the age of the brood was included as a random factor as well, to account for the variance due to differences in growing speed amongst age periods (Turner, 2006).

GLMMs were initially fitted with all explanatory terms included in them, including first-order interactions in which sexual ornaments (tail length, ventral and throat brightness) and physical condition were involved. A summary of the maximal models fitted is given in Table 1. Minimal adequate models were chosen after a process of stepwise deletion of the least significant effects ($p > 0.05$), starting with the highest-order interactions, providing the simplification did not significantly reduce the explanatory power of the model (Crawley, 2002; Schuett & Dall, 2010). Consecutive models were compared using chi-square (χ^2) statistics. If a factor containing several levels had a significant effect in the minimal model, we applied *post-hoc* pairwise comparisons between the levels of the factor using the *glht* function and Tukey's test of additivity from the *multcomp* extension package (Hothorn, Bretz & Westfall, 2008) of R 2.13.0 (R Development Core Team, 2010). These pairwise comparisons allowed us to test the significance of the effect of each factor level compared to the rest of the levels (Table 1). The residuals from the models were checked for normality and homocedasticity; where appropriate, transformations of the response variables or standardizations of the explanatory variables were done following R Development Core Team (2010).

Results

No variable or interaction analysed was found to have a significant effect on clutch size for the first clutch, when this was tested as the response variable (Table 1).

Our analyses showed a significant two-way interaction between sex and throat brightness on hatching success, i.e. the proportion of eggs laid which successfully hatched, for the first clutch (χ^2_1=4.911, p=0.027; Table 1). Eggs laid by females mated to males with darker throats showed a significantly higher hatching success than those of females mated to males with more brightly coloured throats. We could not find a similarly clear pattern in the case of females' throat colouration (Figure 1). The analyses also showed a significant 2-way interaction between ventral brightness and throat brightness on hatching success (χ^2_1=9.450, p=0.002, Table 1). In general, individuals with brighter throat plumage (males or females) had clutches with a higher hatching success for all the range of ventral brightness values. Hatching success amongst brighter-throated swallows was higher for those who also had brighter coloured ventral plumage. Amongst darker-throated swallows, however, hatching success was slightly higher for those with darker ventral colouration (Figure 2). Finally for hatching success in first clutch, we found a significant 2-way interaction between tail length and throat brightness on the proportion of successfully hatched eggs (χ^2_1=5.158, p=0.023). Hatching was more successful for swallows with darker throats, especially for those with longer tail streamers. Amongst the swallows with brighter throat colouration, on the other hand, shorter-tailed individuals had a higher hatching success than longer-tailed ones (Figure 3).

When analysing the physical condition of the chicks raised in the first clutch by the barn swallows in our study population, we found it to be significantly determined by the 2-way interaction between ventral brightness and the abundance of large insects around breeding sites (χ^2_1=4.882, p=0.027; Table 1). For swallows nesting in places next to foraging areas with a lower large insect abundance, ventral colouration of the parents did not seem to have any effect on physical condition of the chicks. In places related to foraging areas richer in large insects, however, chicks raised by parents with a brighter ventral plumage colouration were in much better physical condition than those raised by parents with darker ventral plumages (Figure 4).

The analyses carried out for the second clutch yielded a significant effect of the 2-way interaction between ventral plumage colouration (before manipulation) and large insect abundance on clutch size ($\chi^2_1=4.019$, $p=0.045$). Clutches were bigger for couples nesting in sites associated with foraging areas richer in large insects. Females of pairs in which barn swallows had a brighter ventral plumage laid more eggs than those of couples with darker ventral plumage colouration, this difference being much more pronounced for individuals nesting in sites with foraging areas more abundant in large insects (Figure 5). Plumage manipulations also had a significant effect on the size of the second clutch ($\chi^2_6=21.396$, $p=0.002$). Females mated to control males laid significantly fewer eggs than those mated to males with elongated tail streamers ($z_1=-3.478$, $p=0.0005$), reduced tail streamers ($z_1=-3.170$, $p=0.002$), control males with enhanced ventral plumage colouration ($z_1=3.337$, $p=0.0008$) or unmanipulated males ($z_1=3.938$, $p<0.0001$). Likewise, females mated to males with reduced tail streamer length and enhanced ventral plumage colouration laid significantly fewer eggs than those mated to males with elongated tail streamers ($z_1=-2.034$, $p=0.042$), control males with enhanced ventral plumage colouration ($z_1=2.051$, $p=0.040$) or unmanipulated males ($z_1=1.975$, $p=0.048$; Table 1; Figure 6).

There was a significant 2-way interaction between throat brightness before manipulation and the abundance of large insects on the hatching success for the second clutch ($\chi^2_1=15.054$, $p<0.001$; Table 1). Hatching success was higher for couples whose members had brighter throat plumage patches, and this difference was much more pronounced for swallows nesting in sites associated with foraging areas with greater large insect abundance. The highest hatching success was recorded for brighter individuals foraging in sites richer in large insects (Figure 7). Also, the 2-way interaction between ventral plumage colouration before manipulation and large insect abundance had a significant effect on hatching success (χ^2_1=8.971, p=0.003; Table 1). Amongst swallows nesting in sites associated with foraging areas with lower abundance of large insects, those with brighter ventral plumage had clutches that hatched much more successfully than the ones with darker ventral colouration. This relationship reversed for individuals foraging in areas with higher large insect abundance, with darker swallows getting a higher hatching success. The highest hatching success was recorded for brighter individuals nesting in sites with lower large insect abundance, and to a lesser extent, for darker ones nesting next to areas with higher large insect abundance (Figure 8). Furthermore, there was a significant 2-way interaction between throat plumage colouration and tail length (both before manipulation) on hatching success (χ^2_1=15.332, p<0.001; Table 1). Barn swallows with darker throat plumage patches had a much higher hatching success for their second clutches than brighter-throated individuals. Amongst birds with darker throat patches, those with longer tail streamers had a higher proportion of eggs successfully hatched, whereas amongst individuals with brighter throat colouration, short tail streamered ones had a higher hatching success (Figure 9). Again, plumage manipulations had a significant effect on hatching success (χ^2_6=28.293, p<0.001). Clutches from pairs whose males' tail streamers were artificially elongated had significantly higher (and less variable) hatching success than clutches from couples with males' tail streamers reduced (z_1=-4.009, p<0.0001), streamers reduced and ventral colouration enhanced (z_1=-2.535, p=0.011), control males (z_1=-4.997, p<0.0001), unmanipulated males (z_1=-6.465, p<0.0001) or control males with enhanced ventral plumage colouration (z_1=-3.166, p=0.002). Males with artificially shortened tail streamers were part of couples with a significantly higher hatching success and less variance than control (z_1=-2.574, p=0.010) or unmanipulated males (z_1=-2.603, p=0.009). In contrast, control males with enhanced ventral plumage colouration were in couples with significantly lower hatching success than control males (z_1=2.509, p=0.012), or unmanipulated males (z_1=-2.210, p=0.027; Table 1, Figure 10).

When we analysed the physical condition of the chicks in the second clutch, there was a significant 2-way interaction between sex and physical condition of the adults before manipulation on the response variable (χ^2_1=8.154, p=0.004; Table 1). Chicks raised by couples in which males were in better condition were also in much better condition. We did not detect a similar relationship with females' condition (Figure 11). The 2-way interaction between throat plumage brightness before manipulation and sex also had an effect on the physical condition of the chicks (χ^2_1=11.486, p<0.001; Table 1). Chicks raised in nests with darker-throated males were in significantly better condition than those raised by males with brighter throat patches. We observed the opposite trend in females, but it was not significant (Figure 12). Our analyses showed a significant 2-way interaction between tail streamer length before manipulation and sex on chicks' physical condition (χ^2_1=4.140, p=0.042; Table 1). Males with originally longer tail streamers were part of couples that raised chicks in significantly better physical condition. We didn't see any comparable relationship for females' tail streamer length (Figure 13). The 2-way interaction between ventral plumage colouration (before manipulation) and large insect abundance around breeding sites also had an influence on physical condition of chicks (χ^2_1=18.391, p<0.001; Table 1). Physical condition was much better for chicks raised by brighter individuals. Amongst darker barn swallows, those nesting in sites next to foraging areas with a lower large insect abundance had chicks in a better condition (Figure 14). Further to that, there was a significant 2-way interaction between tail streamer length before manipulation and large insect abundance around breeding sites on physical condition of chicks (χ^2_1=11.142, $p<0.001$; Table 1). Chicks raised in nests related to foraging areas with a lower large insect abundance were generally in better condition. Both for individuals next to areas with lower or higher large insect abundance, couples whose male's tail streamers were longer had chicks in better condition (Figure 15). A significant 2-way interaction between ventral plumage colouration and tail length, both before manipulation, on chick condition was found in our study population (χ^2_1=38.107, $p<0.001$; Table 1). Darker individuals raised chicks in better physical condition than those with brighter ventral plumage patches. In the case of brighter barn swallows, those couples whose males had shorter tail streamers raised chicks in slightly better condition (Figure 16). Finally, plumage manipulation had a significant influence on physical condition of chicks (χ^2_5=49.483, $p<0.001$). Couples in which males' tail streamers were artificially elongated raised chicks in significantly poorer physical condition than couples with control males (z_1=8.644, $p<0.0001$), control males with enhanced ventral plumage colouration (z_1=-3.646, $p=0.0003$) or unmanipulated males (z_1=12.710, $p<0.0001$). Furthermore, chicks born in nests where males' tail streamer lengths were shortened were also in worse physical condition than those born in nests with control males (z_1=9.879, $p<0.0001$), control males with enhanced ventral colouration (z_1=-4.153, $p<0.0001$) or unmanipulated males (z_1=22.588, $p<0.0001$). Couples whose males were assigned to the control group and their ventral plumage colouration enhanced raised chicks in better condition than couples with control (z_1=-11.469, $p<0.0001$) or unmanipulated (z_1=19.893, $p<0.0001$) males. Finally, chicks raised in nests with control males were in worse physical condition than those raised in nests with unmanipulated males (z_1=-3,244, p=0.001; Table 1, Figure 17).

Discussion

The results of our study show that throat and ventral colouration-related ornaments, further to tail streamer length, are part of the European barn swallow’s multi-component signalling system, and influence reproductive success. Darker-throated males were in pairs with significantly higher hatching success in the first clutch, and raised chicks in significantly better physical condition in the second clutch. Furthermore, male tail streamer length before manipulation along with male physical condition, were positively related to chick condition in the second clutch. Thus, darker-throated or longer-tailed males appeared to raise chicks in inherently better condition. This suggests that both traits, elongated tail streamers and throat plumage colouration, qualify as potential honest quality-indicating sexual ornaments (Andersson, 1994). While several previous studies have pointed at the quality-indicating function of tail streamers and their relationship with annual reproductive success (Møller, 1994, 1998b), our results are the first, to the best of our knowledge, to demonstrate this for throat plumage colouration (but see Ninni *et al.*, 2004, for indirect evidence through arrival date).

Throat plumage colouration, together with ventral plumage colouration, along with tail streamer length, also explained variation in reproductive success in interaction with other variables. The interaction between throat plumage colouration and tail length influenced hatching success in both clutches. In both cases, individuals with darker throat patches and longer tail streamers had the highest proportion of eggs in the clutch successfully hatched, with darker-throated, shorter-tailed ones also achieving notably enhanced hatching success. This observation confirms our hypothesised quality-indicating value of plumage colouration, either by itself or together with tail streamer exaggeration. The fact that we did not observe significant sex differences for this interaction might suggest that these ornaments are also subject to sexual selection in females to a certain extent, rather than being just nonadaptive, correlated effects of selection on males (Amundsen, 2000). Although the studies demonstrating functional female ornaments are rare for birds, some previous work has shown enhanced fecundity in the most exaggeratedly ornamented females (Møller, 1993; Safran & McGraw, 2004). Likewise, throat plumage darkness in males alone positively influencing first clutch hatching success might be indicative of differential maternal allocation in relation to male sexiness (Burley, 1986; Osorno et al., 2006; Loyau & Lacroix, 2010). Ventral plumage colouration, interacting with tail length, also influenced physical condition of chicks during the second clutch. Again, individuals with darker plumage patches achieved the higher values of reproductive success by raising chicks in much better condition. For individuals with darker ventral plumage, tail streamer length hardly had any effect on the physical condition on chicks. Amongst brighter-coloured ones, though, shorter-tailed barn swallows raised chicks in better condition than longer-tailed ones. This may suggest a higher importance of "cheaper" (colour-related) sexual signals rather than more "expensive" ones (elongated tail streamers) as quality-indicators in our study population, possibly due to geographic variation in environmental conditions (Safran & McGraw, 2004): if individual quality is mainly signalled by plumage colouration, we would expect brighter individuals with longer tail streamers to find it more difficult to capture appropriate prey items due to aerodynamic constraints (Møller & de Lope, 1994). This would ultimately have repercussions on chick physical condition.

For first clutches, the interaction between throat and ventral plumage colouration on hatching success showed this to be higher for pairs whose members had brighter coloured plumage patches (both throat and ventral). Evidence for a “compensation mechanism“ has been found in this European barn swallow population, which would help explain the persistence of relatively high variance in the phenotypic expression of secondary sexual characters that are part of potential multi-component signalling systems (Vaquero-Alba *et al.*, unpublished data; **3**). According to this evidence, individuals with lower investment in one or more ornaments involved in sexual signalling should be selected to seek and defend breeding sites associated with foraging sites richer in large insects (the most profitable prey for barn swallows; Turner, 2006). If hatching success increases with large insect abundance, this mechanism might help explain, at least partly, why the highest hatching success was observed for individuals with brighter plumage.

Actually, large insect abundance around breeding sites, in interaction with other variables, explained some of the reproductive success indicators we measured. During the first clutch, chicks in the best physical condition were raised by brighter parents nesting in sites surrounded by areas richer in large insects. Likewise, (originally) brighter parents also raised chicks in better condition in the second clutch, but this time no difference was detected between sites associated with foraging areas with lower or higher large insect abundance. Lower-quality individuals, in terms of ventral plumage colouration, seemed to compensate by feeding their chicks at a higher rate in the first clutch, but only in sites where a greater abundance of large insects permitted it. Paradoxically, in the second clutch, amongst darker individuals, those birds foraging in areas poorer in large insects were the ones that had chicks in better condition. These results, contrary to our initial prediction, suggest that parents with a higher investment in ventral colouration provide less parental care than those with lower investment, as it has been reported in previous studies for males in relation to tail streamer length (de Lope & Møller, 1993; Møller *et al.*, 1998a). It remains to be tested whether plumage darkness, even as a less costly signal (at least to maintain) than elongated tail streamers, is imposing any kind of limit on foraging efficiency, which would help explain our observations. The condition of chicks in second clutches was also influenced by tail streamer length in interaction with large insect abundance. Counterintuitively, chicks in better condition were raised by individuals nesting next to areas with fewer large insects, especially those with longer tail streamers. Whether tail streamer length exaggeration is mainly due to sexual selection (Møller, 1994) or only partly (Evans, 1998), higher quality individuals in terms of tail streamer length should raise chicks in better physical condition due to their heritable quality (Zahavi & Zahavi, 1997). Furthermore, the "compensation mechanism" mentioned above would help explain the presence of higher-indicator/investing swallows in sites associated to lower large insect abundance (Vaquero-Alba *et al.*, unpublished data; **3**).

Also, during the second clutch, when analysing the interaction between ventral plumage brightness and large insect abundance on clutch size, we observed that larger clutches were recorded for brighter individuals, especially those nesting in sites associated with areas richer in large insects. Thus, females with lower investment in ventral plumage colouration and/or mated to lower-investing males might be selected to allocate more resources to laying a larger amount of eggs, perhaps to compensate for their lack of fitness compared to darker individuals. This higher investment in producing more eggs, however, was not reflected in hatching success, which was higher for brighter individuals nesting in sites close to areas with lower large insect abundance, and for darker individuals nesting in sites associated with areas richer in large insects.

Hunt (1973) reported decreased hatching success, as well as an eggshell-thinning, for eggs in unusually large clutches in western gulls. Females of a lower quality and/or mated to lower quality males for the character considered (i.e. with brighter ventral plumage) that invested more in egg production, may have experienced a cost in terms of lower hatching success. This, however, would not explain the increased hatching success for brighter individuals in areas less abundant in large insects. As we are studying a multi-component signalling system, it would not be surprising if these individuals were actually of better quality than is apparent from this character alone, due to more investment in alternative ornaments. When considering the interaction between throat plumage colouration and large insect abundance on hatching success for the second clutch, higher hatching success was found for brighter-throated individuals nesting in sites associated with areas richer in large insects, but also, to a lesser extent, brighter ones nesting next to areas poor in large insect abundance. These results might be due, again, to these individuals being of better quality than the analysis of this single interaction reveals, as other interactions analysed clearly suggest a positive influence of exaggerated sexual ornaments on reproductive success.

In accordance with previous studies, we found, in most cases, that pairs in which male tail streamers were artificially lengthened had higher and less variable hatching success than pairs in which males were assigned to any other experimental group. Evidence suggests that females can affect the quality of eggs by supplying them with carotenoids and other substances that can improve the development of the embryonic immune system (Turner, 2006), which would lead to an enhanced hatching success. Saino *et al.* (2002b) reported a higher transfer of antibodies to first-laid eggs from vaccinated barn swallow females when mated to artificially long-tail, i.e. more attractive-looking, males. In this study, males with experimentally reduced tail streamers were in pairs with higher and less variable hatching success than control or unmanipulated males. Females barn swallows mated to experimentally short-tailed males transfer more lutein to the eggs than those mated to experimentally long-tailed ones, to compensate for the poor parasite resistance of the chicks of poor quality (shorter-tailed) males (Saino *et al.*, 2002a). Due to luteins' antioxidant properties, we would expect this transfer to increase hatching success.

Also in accordance with existing evidence, the results we observed for the effect of plumage manipulation on physical condition of chicks can be explained in terms of differential aerodynamic costs for males subject to tail streamer elongation or shortening and differential female reproductive effort according to the degree of ornamentation of their males. Males with experimentally elongated tails undergo an increased flight cost during aerial feeding. Thus, they capture a higher proportion of smaller prey items, which is energetically inefficient (Møller *et al.*, 1995; Turner, 2006). Although it has been reported that females mated to experimentally longer-tailed males adjust their feeding behaviour in order to compensate for impaired male foraging ability (de Lope & Møller, 1993), our study suggests that this compensation may not be enough: chicks raised by such pairs were in significantly worse physical condition than those raised by control males, with or without plumage colour enhancement, or unmanipulated males, and their mates. Females mated to experimentally short-tailed males may also have adjusted their feeding behaviour to being with an apparently lower-quality (but better foraging) mate, by reducing their foraging effort. This would help explain why, despite males within these couples being able to capture more profitable prey items (Møller *et al.*, 1995) their chicks are in poorer condition than those raised by control males, with or without plumage colour enhancement, or unmanipulated males, and their mates.

Fitze & Richner (2002) found a significant decrease in the size of the breast stripe of great tits experimentally infested with parasites compared to uninfested ones. They suggested that this trait serves as an honest signal of quality, as predicted by models of sexual selection. On the other hand, Møller (1991) found smaller second clutches for female barn swallows with mite-infested first clutch nests. So, it seems that female barn swallows can adjust their investment in egg production during the second clutch in order to maximise their reproductive success. Specifically, they should adjust it to the ability of both parents to feed their broods (Turner, 2006). Our results show significantly smaller clutch sizes for females mated to males with experimentally short tail streamers and enhanced ventral plumage colouration. If melanin-based ventral plumage colouration is an honest quality signal, we would predict males with experimentally enhanced ventral darkness to have a higher amount of parasites than expected from their external appearance. If females can assess the quality of their mates throughout the season (de Lope & Møller, 1993) and use this information to adjust their investment in clutch size, we would expect them to decrease the production of eggs in response to a "dishonest" male and/or one made to look unattractive. This is exactly what we observed. It is important to point out that only females mated to males in which with both of these characteristics decreased their egg production. We did not observe the same response in females mated to males with only one of these "weaknesses". Thus, this observation highlights not only the honesty of ventral plumage colouration as a sexual signal, but also the multi-component nature of the sexual signalling system in the barn swallow. Clutch size was also significantly lower for females mated to control males than for females mated to males assigned to other experimental groups. As clutch size is not significantly lower for females mated to unmanipulated males, we could argue that the manipulation of the tail itself exerted some negative effect on their bearers and prompted a decrease in investment by the females mated to them. But in that case, we should expect to see a similar response in other manipulation groups, and we did not. An alternative explanation might be that, despite the random assignment of males to experimental groups, those assigned to the control group may be, by chance, of a significantly lower quality than the ones assigned to other groups. Couples with control and ventral colouration-enhanced males had significantly lower hatching success than control or unmanipulated groups, perhaps due to the negative adjustment of female investment to a dishonest quality signal. They also had chicks in significantly better condition.

To sum up, throat and ventral plumage colouration, together with tail streamer length, seem to function as notable quality indicators in the multi-component signalling system of the European barn swallow. We provide evidence, for the first time in this subspecies, that both throat and vent plumage colour patches, in combination with tail length, influence reproductive success, in a similar way to that reported for the North American subspecies. Generally speaking, swallows with higher investment in darker plumage patches, alone or in interaction with tail streamer length or physical condition, seemed to exhibit higher reproductive success. Interactions with habitat quality (i.e. large insect abundance in foraging areas), however, seemed to somehow "reverse" these effects, suggesting the existence of a "compensation" mechanism through habitat selection by individuals with poorer phenotypes, as has been proposed recently. Finally, males with artificially lengthened tail streamers seemed to stimulate higher reproductive investment in the females mated to them, as expected, but plumage colour manipulations did not seem to have any notable effect in this system.

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Table 1 Summary of minimal adequate linear mixed-effect models

Response variable	**N**	**ΔAIC**	**Explanatory variable**	**χ^2(df)**	**p**	**z *ph*(df)**	**p *ph***
(a) First clutch							
Clutch size^{a†}	83	28.99	Sex	0.04 (1)	0.842		
			Condition	0.062 (1)	0.803		
			Tail length	0.129 (1)	0.72		
			Ventral bright	0.211 (1)	0.646		
			Large insects	0.46 (1)	0.498		
			Throat bright	0.48 (1)	0.489		
Hatching success^{a}	83	10	Sex	0.139 (1)	0.709		
			Throat bright	0.182 (1)	0.670		
			Ventral bright	2.735(1)	0.098		
			Tail length	0.389 (1)	0.533		
			Large insects	32.91 (1)	<0.0001		
			Sex (male):throat bright	4.911 (1)	0.027		
			Ventral bright:throat bright	9.450 (1)	0.002		
			Tail length:throat bright	5.158 (1)	0.023		
Chick condition^{b}	86	18.5	Large insects	2.070 (1)	0.150		
			Ventral bright	7.004 (1)	0.008		
			Ventral bright:large insects	4.882 (1)	0.027		
(b) Second clutch (manipulations)							
Clutch size^{c†}	85	23.6	Plumage manip	21.396 (6)	0.002		
			RC-E			-2.034 (1)	0.042
			RC-SC			2.051 (1)	0.040
			RC-U			1.975 (1)	0.048
			S-E			-3.478 (1)	0.0005
			S-R			-3.170 (1)	0.002
			S-SC			3.337 (1)	0.0008
			S-U			3.938 (1)	<0.0001
			Ventral bright	5.584 (1)	0.018		
			Large insects	0.964 (1)	0.326		
			Ventral bright:large insects	4.019 (1)	0.045		
Hatching success^{c}	65	10	Plumage manip	28.293 (6)	<0.0001		
			E-R			-4.009 (1)	<0.0001
			E-RC			-2.535 (1)	0.011
			E-S			-4.997 (1)	<0.0001
			E-SC			-3.166 (1)	0.002
			E-U			-6.465 (1)	<0.0001
			R-S			-2.574 (1)	0.010
			R-U			-2.603 (1)	0.009
			SC-S			2.509 (1)	0.012
			SC-U			-2.210 (1)	0.027
			Throat bright	4.654 (1)	0.031		
			Ventral bright	18.204 (1)	<0.0001		
			Tail length	2.052 (1)	0.152		
			Large insects	22.176 (1)	<0.0001		
			Throat bright:large insects	15.054 (1)	<0.0001		
			Ventral bright:large insects	8.971 (1)	0.003		
			Throat bright:tail length	15.332 (1)	<0.0001		

Chick condition[d ‡]	50	7.7	Plumage manip	49.483 (5)	<0.0001		
			E-S			8.644 (1)	<0.0001
			E-SC			-3.646 (1)	0.0003
			E-U			12.710(1)	<0.0001
			R-S			9.879 (1)	<0.0001
			R-SC			-4.153 (1)	<0.0001
			R-U			22.588 (1)	<0.0001
			SC-S			-11.469 (1)	<0.0001
			SC-U			19.893 (1)	<0.0001
			S-U			-3.244 (1)	0.001
			Sex	0.0004 (1)	0.992		
			Condition	0.173 (1)	0.678		
			Tail length	0.170 (1)	0.68		
			Throat bright	1.773 (1)	0.183		
			Ventral bright	0.655 (1)	0.418		
			Large insects	0.090 (1)	0.764		
			Sex (male):condition	8.1535 (1)	0.004		
			Sex (male):throat bright	11.486 (1)	0.0007		
			Sex (male):tail length	4.1402 (1)	0.042		
			Ventral bright:large insects	18.391 (1)	<0.0001		
			Tail length:large insects	11.142 (1)	0.0008		
			Ventral bright:tail length	38.107 (1)	<0.0001		

ΔAIC difference between AIC of maximal and minimal model, *condition* physical condition, covariate, *large insects* large insect abundance, covariate, *measureday* day of measurement, factor with 5 levels, *nest:site* nest location within each site, factor with 37 levels, *plumage manip* plumage manipulation, factor with 7 levels, *E* tail elongation, *EC* tail elongation+colour enhancement, *R* tail reduction, *RC* tail reduction+colour enhancement, *S* control, *SC* control+colour enhancement, *U* unmanipulated, *sex* factor with 2 levels, *site* geographic location of nesting site, factor with 12 levels, *tail length* tail length, covariate, *throat bright* throat brightness, covariate, *ventral bright* ventral brightness, covariate, *year* factor with 2 levels, *z ph* z score for each pairwise comparison after post-hoc analyses.

[a] Fixed effects and covariates included in maximal models: sex, large insects, tail length, throat bright, ventral bright, condition, sex:tail length, sex:throat bright, sex:ventral bright, sex:condition, large insects:tail length, large insects:throat bright, large insects:ventral bright, large insects:condition, tail length:condition, throat bright:condition, ventral bright:condition, tail length:throat bright, tail length:ventral bright, ventral bright:throat bright; random effects: site/nest, year.

[b] Fixed effects and covariates included in maximal models: sex, large insects, tail length, throat bright, ventral bright, condition, sex:tail length, sex:throat bright, sex:ventral bright, sex:condition, large insects:tail length, large insects:throat bright, large insects:ventral bright, large insects:condition, tail length:condition, throat bright:condition, ventral bright:condition tail length:throat bright, tail length:ventral bright, ventral bright:throat bright; random effects: site/pair, year, measureday.

[c] Fixed effects and covariates included in maximal models: sex, plumage manip, large insects, tail length, throat bright, ventral bright, condition, sex:tail length, sex:throat bright, sex:ventral bright, sex:condition, large insects:tail length, large insects:throat bright, large insects:ventral bright, large insects:condition, tail length:condition, throat bright:condition, ventral bright:condition, tail length:throat bright, tail length:ventral bright, ventral bright:throat bright; random effects: site/nest, year.

[d] Fixed effects and covariates included in maximal models: sex, plumage manip, large insects, tail length, throat bright, ventral bright, condition, sex:tail length, sex:throat bright, sex:ventral bright, sex:condition, large insects:tail length, large insects:throat bright, large insects:ventral bright, large insects:condition, tail length:condition, throat bright:condition, ventral bright:condition, tail length:throat bright, tail length:ventral bright, ventral bright:throat bright; random effects: site/nest, year, measureday.

[†] Explanatory variables were standardized: stand(x)=x-mean(x))/sd(x)

[‡] Response variable was gamma transformed

Type of manipulation	Description
E	Tail streamer elongation
EC	Tail streamer elongation + colour enhancement
R	Tail streamer reduction
RC	Tail streamer reduction + colour enhancement
S	Control
SC	Control + colour enhancement
U	Unmanipulated

Table 2: Description of the levels in the "plumage manipulation" factor

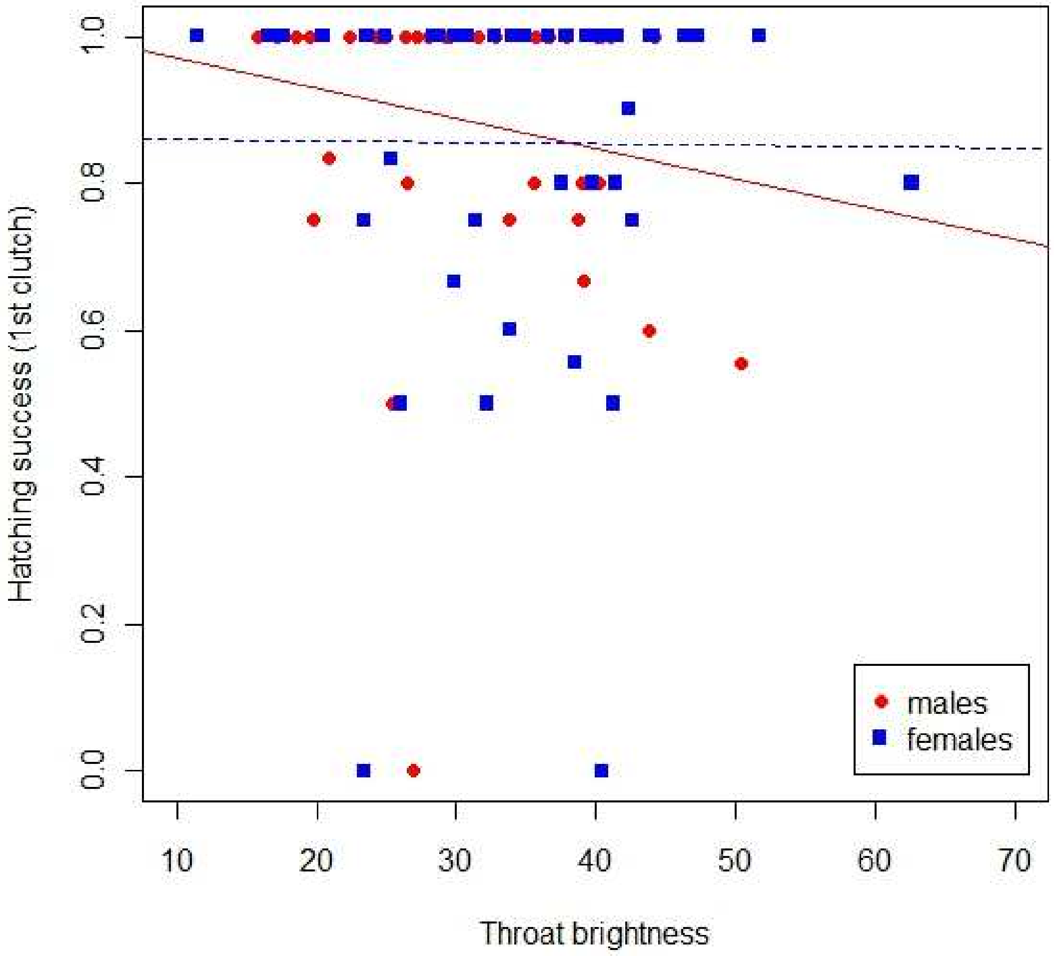

Figure 1: Effect of throat brightness on hatching success

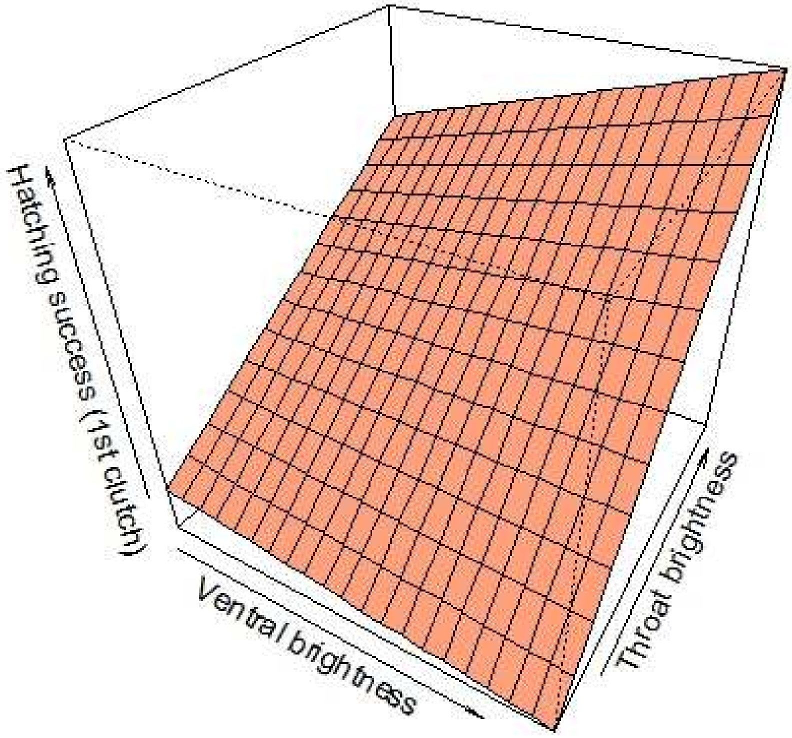

Figure 2: Effect of throat and ventral brightness on hatching success - 1st clutch (Model fit)

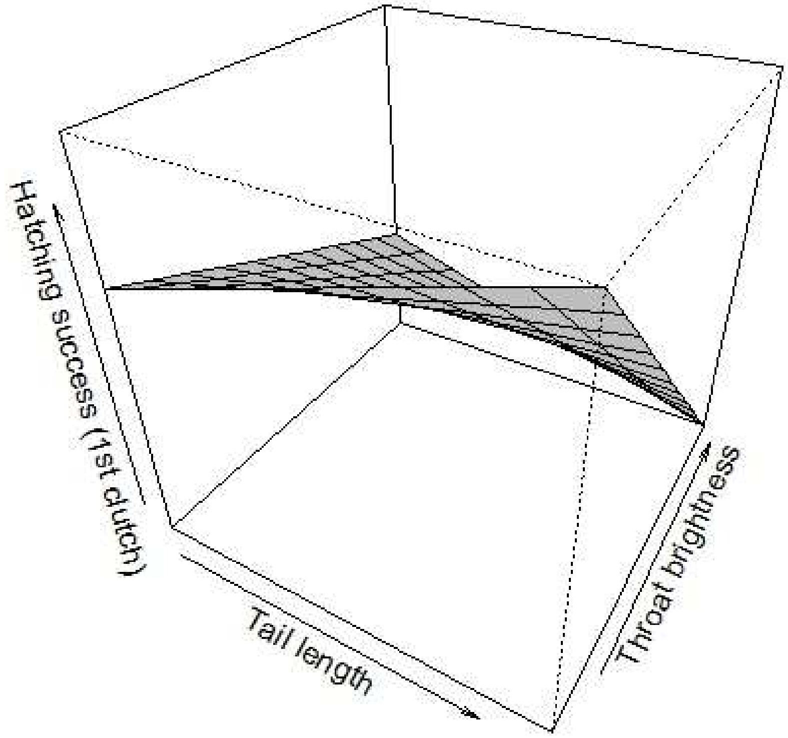

Figure 3: The effect of throat brightness and tail length on hatching success - 1st clutch (Model fit)

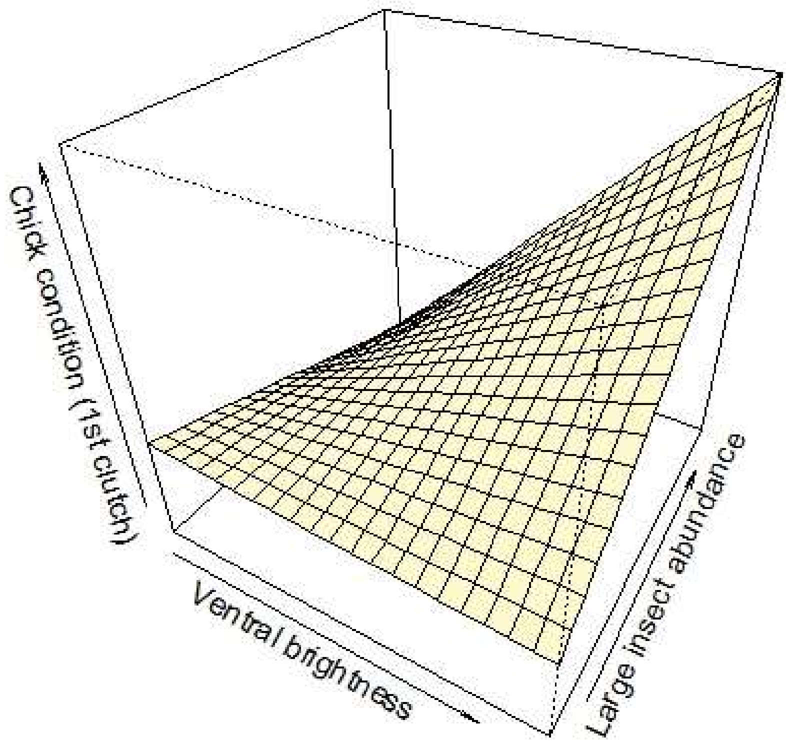

Figure 4: The effect of ventral brightness and large insect abundance on hatching success - 1st clutch (Model fit)

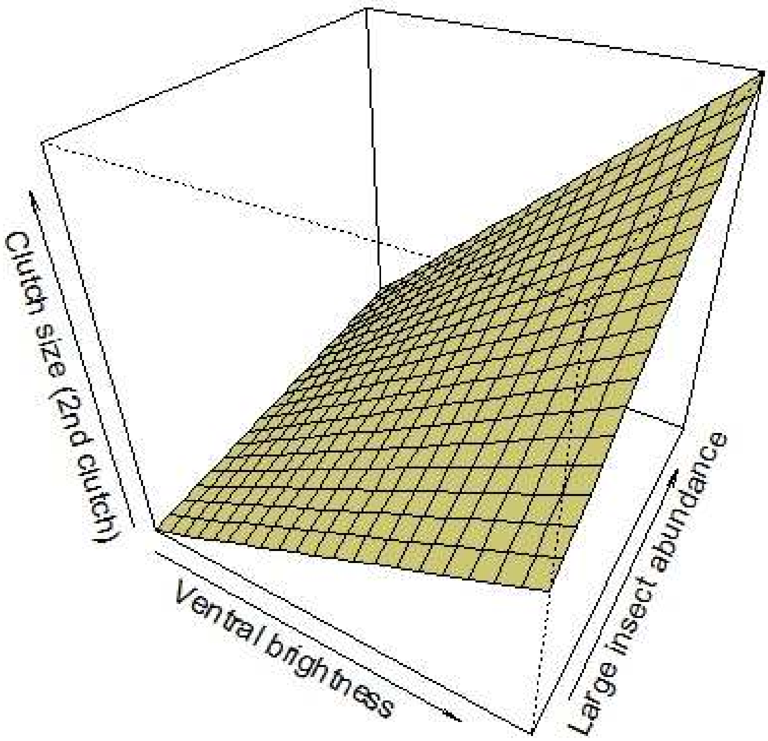

Figure 5: The effect of ventral brightness and large insect abundance on clutch size - 2^{nd} clutch (Model fit)

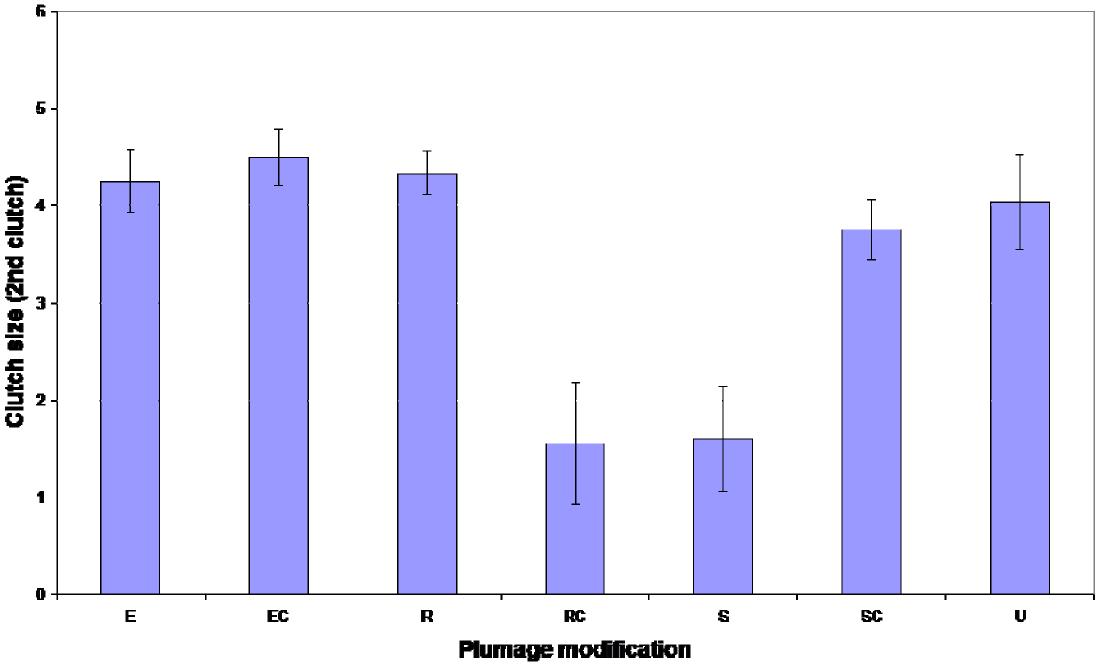

Figure 6: Effect of plumage manipulation on clutch size (2nd clutch).

(For a description of levels of "plumage modification", see Table 2)

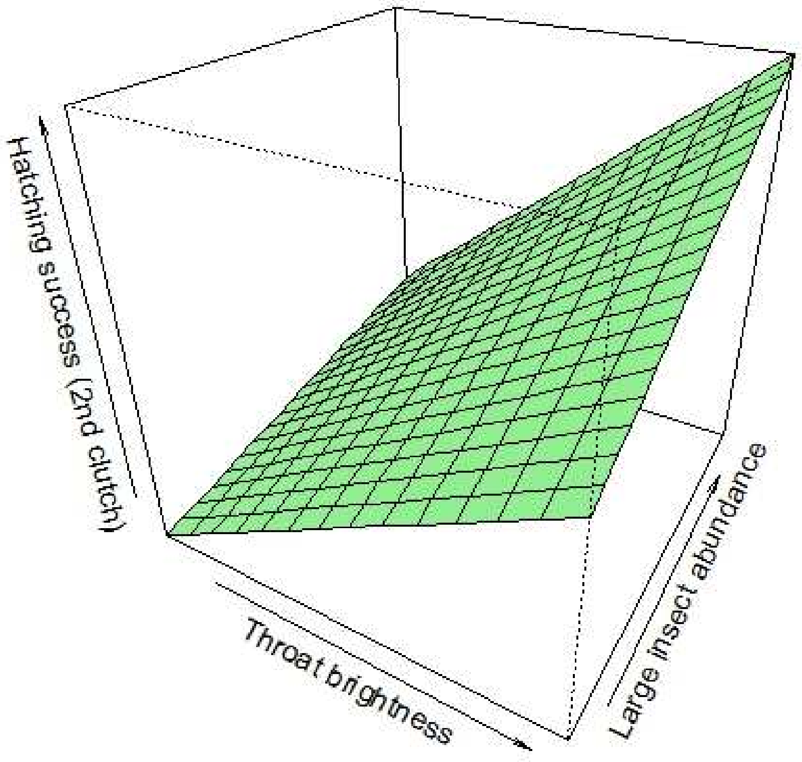

Figure 7: The effect of throat brightness and large insect abundance on hatching success - 2nd clutch (Model fit)

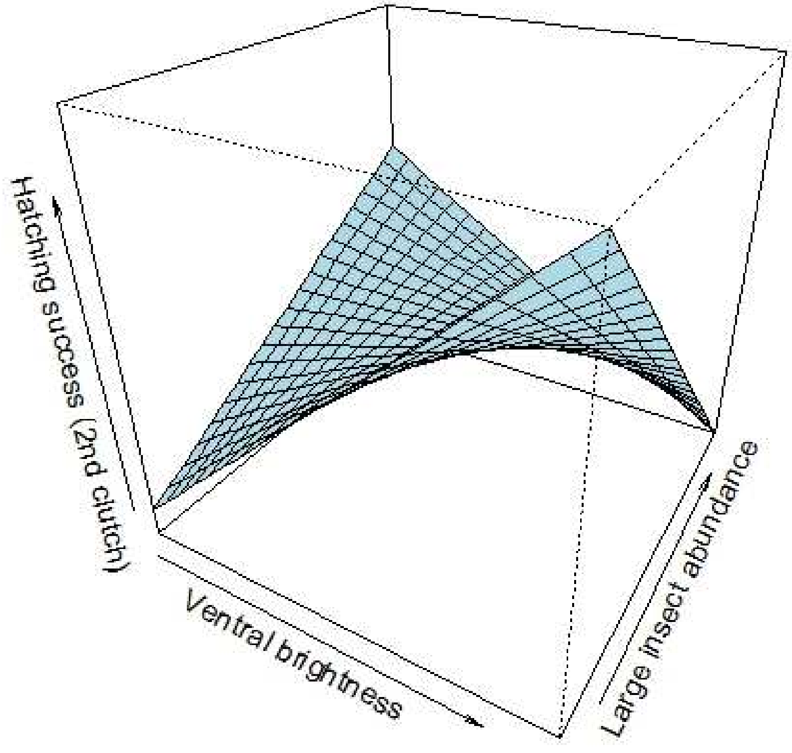

Figure 8: The effect of ventral brightness and large insect abundance on hatching success - 2nd clutch (Model fit)

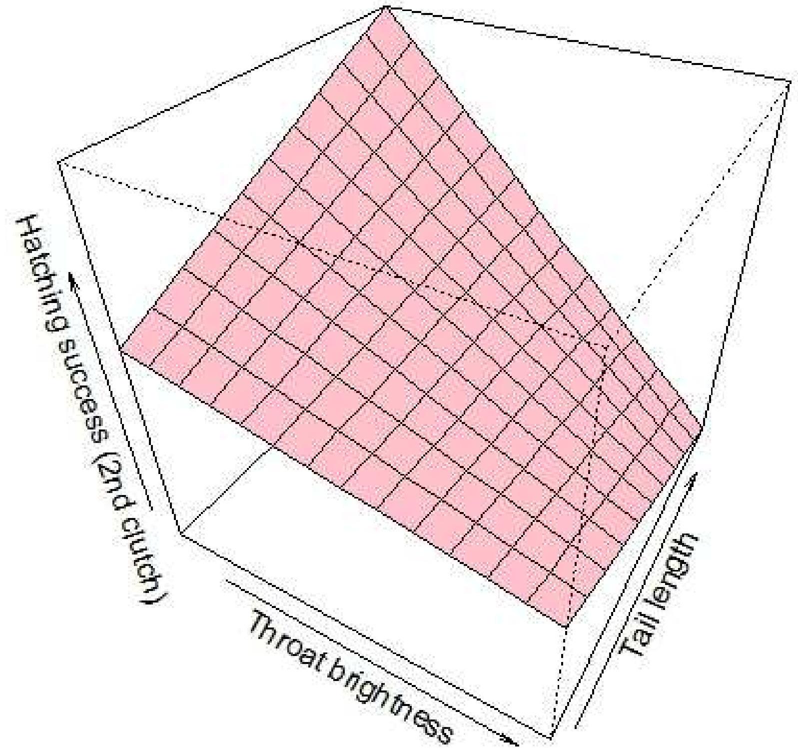

Figure 9: The effect of throat brightness and tail length on hatching success - 2nd clutch (Model fit)

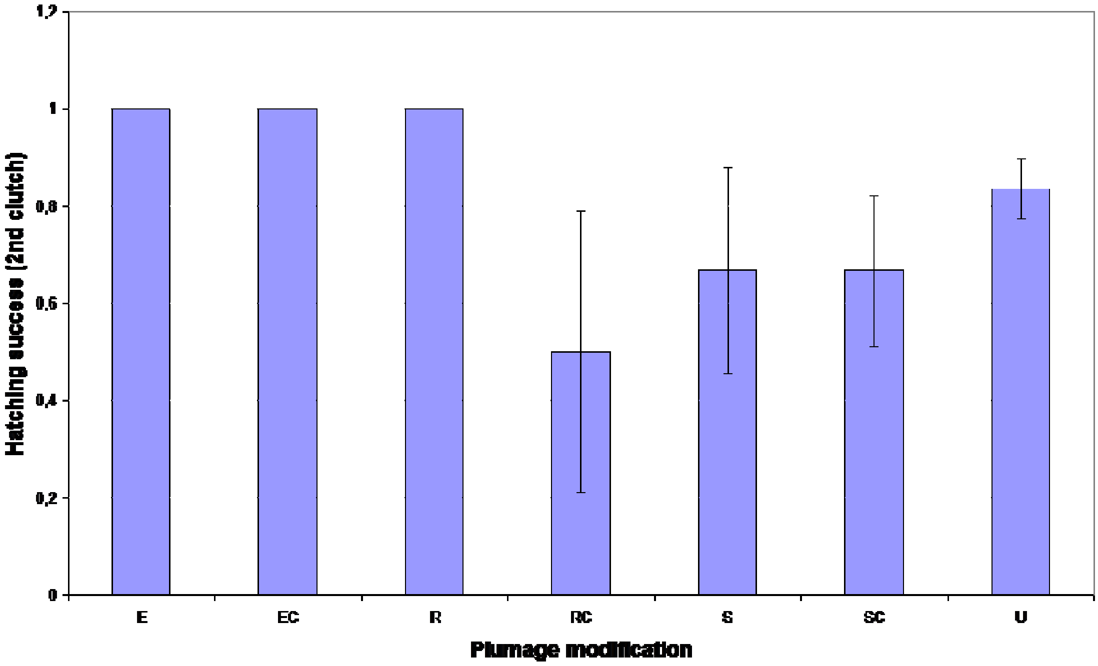

Figure 10: Effect of plumage manipulation on hatching success (2nd clutch).

(For a description of levels of “plumage modification”, see Table 2)

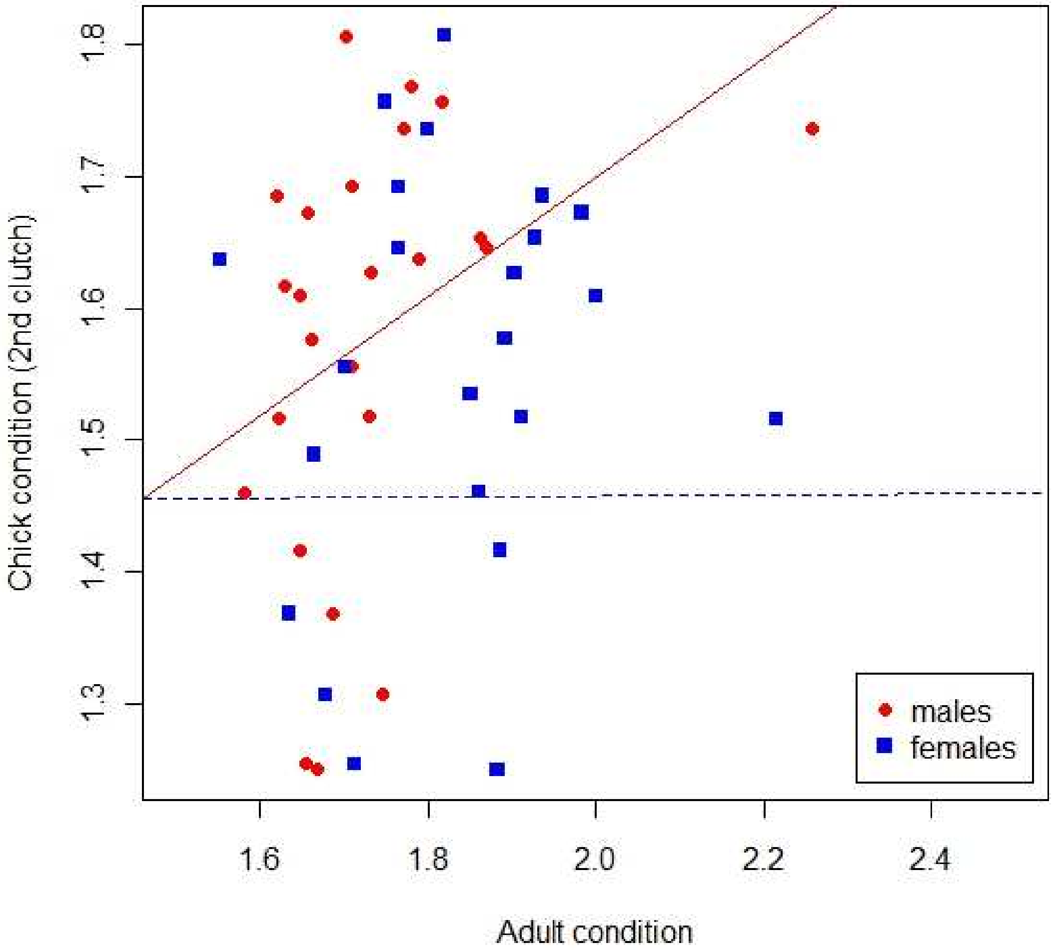

Figure 11: Effect of adult condition on chick physical condition (2nd clutch)

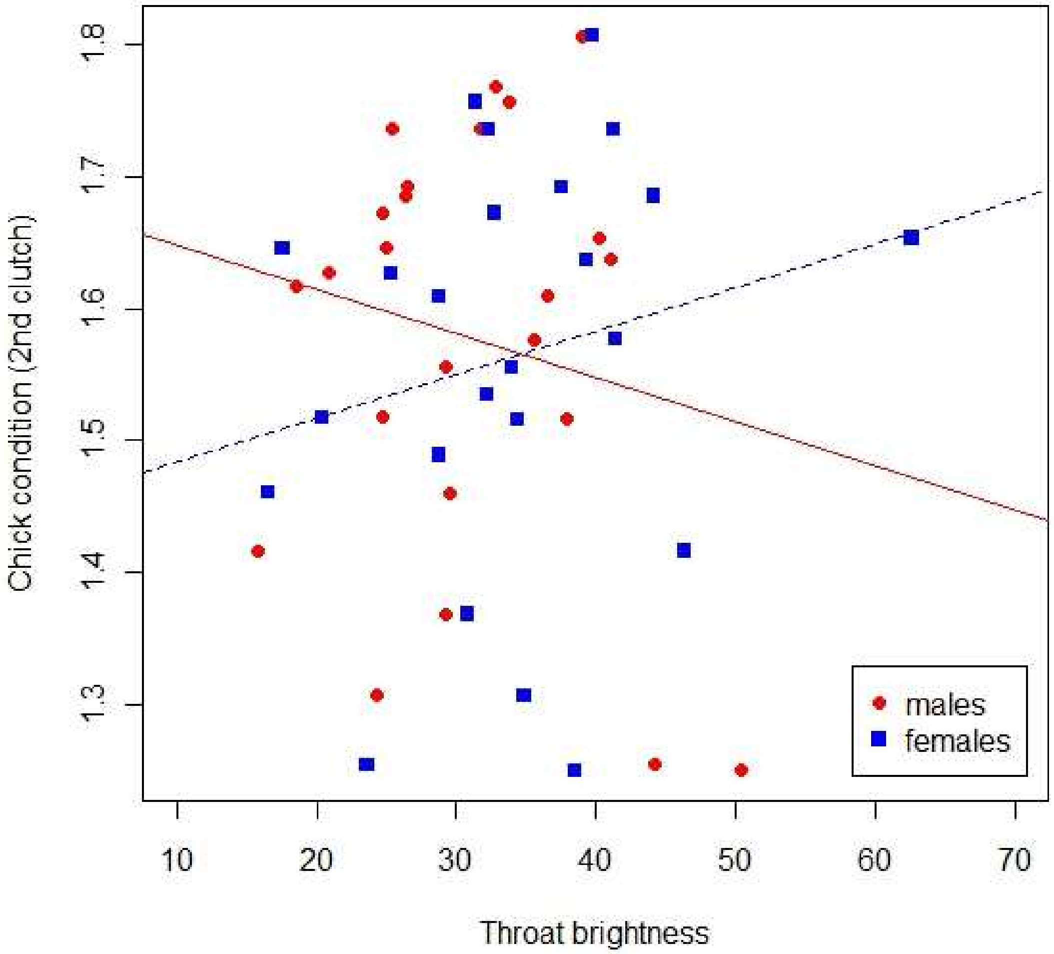

Figure 12: Effect of throat brightness on chick physical condition (2^{nd} clutch)

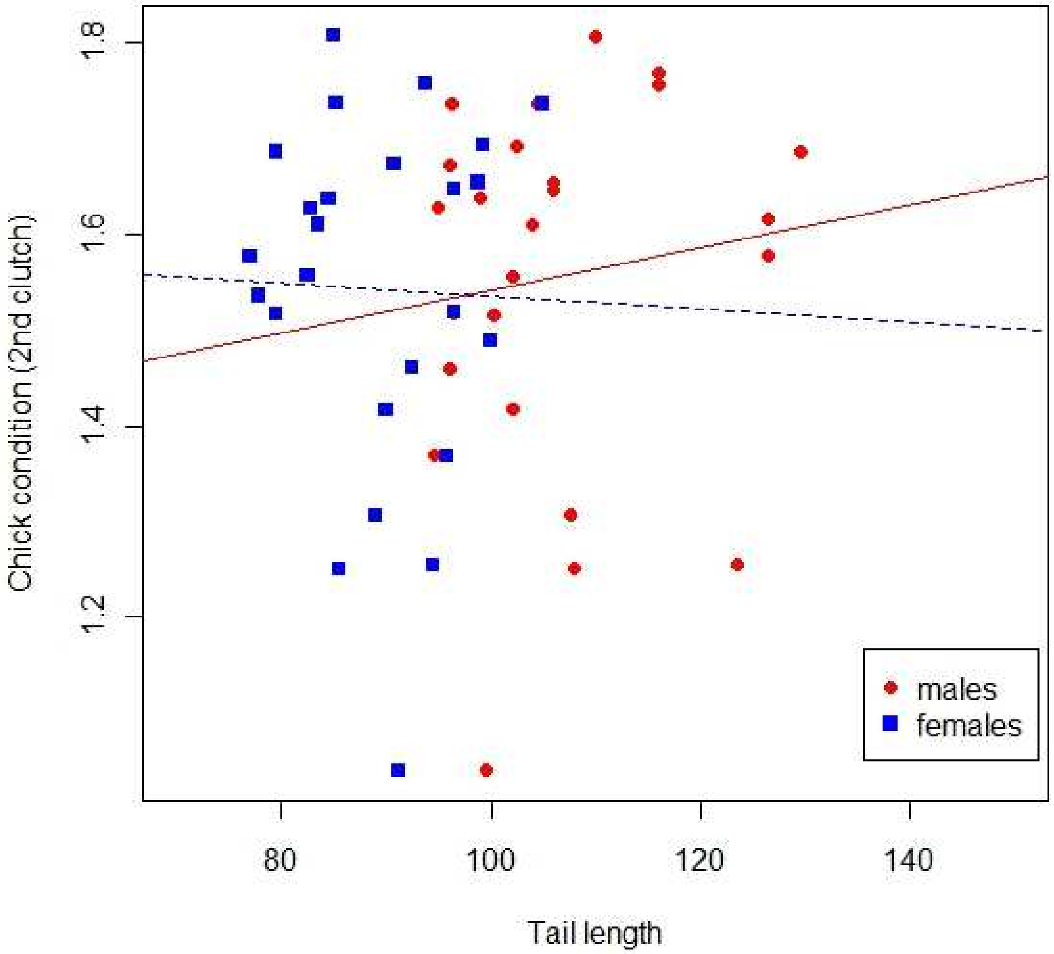

Figure 13: Effect of tail streamer length on chick physical condition (2^{nd} clutch)

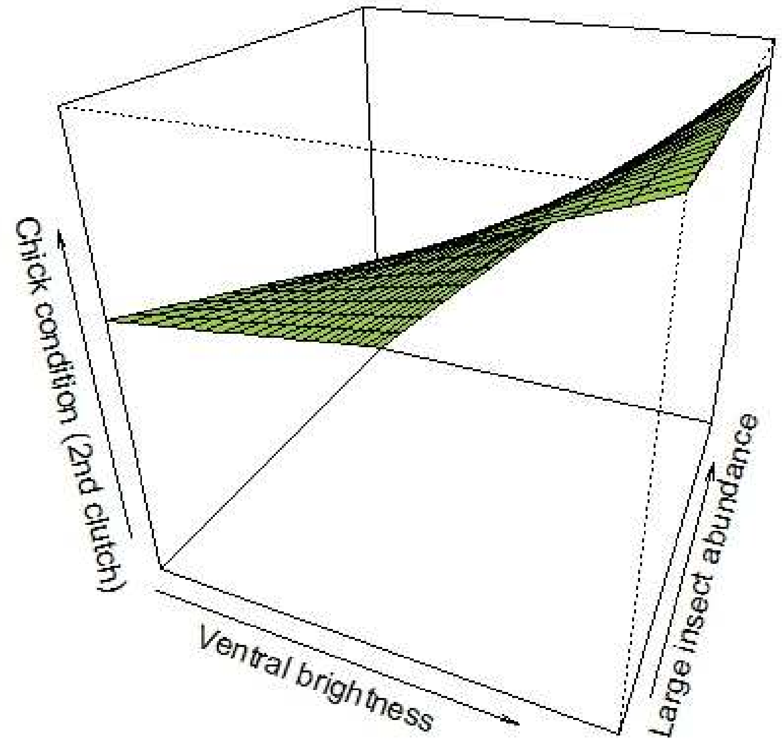

Figure 14: The effect of ventral brightness and large insect abundance on chick physical condition - 2^{nd} clutch (Model fit)

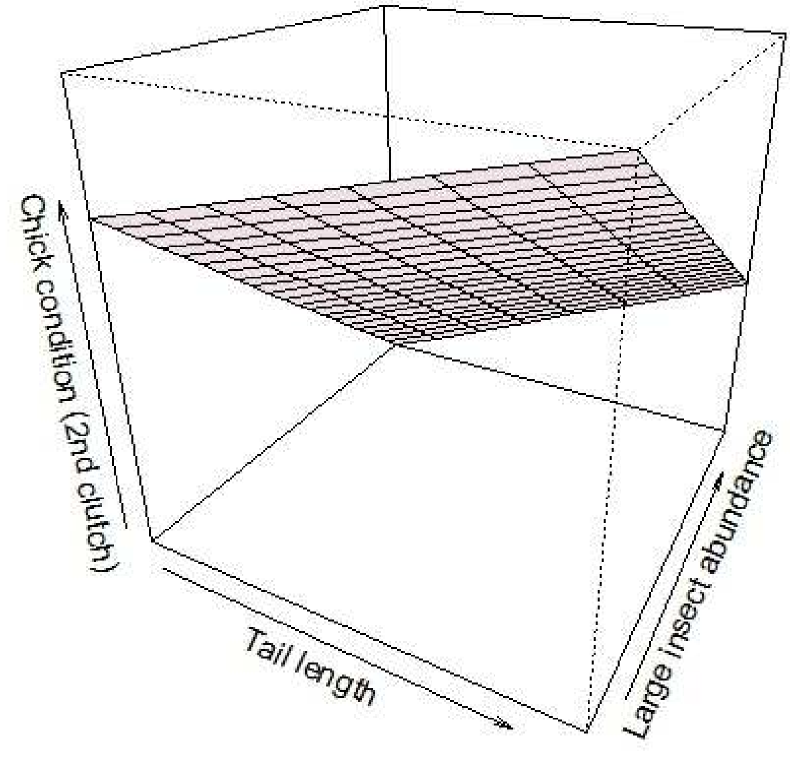

Figure 15: The effect of tail length and large insect abundance on chick physical condition - 2^{nd} clutch (Model fit)

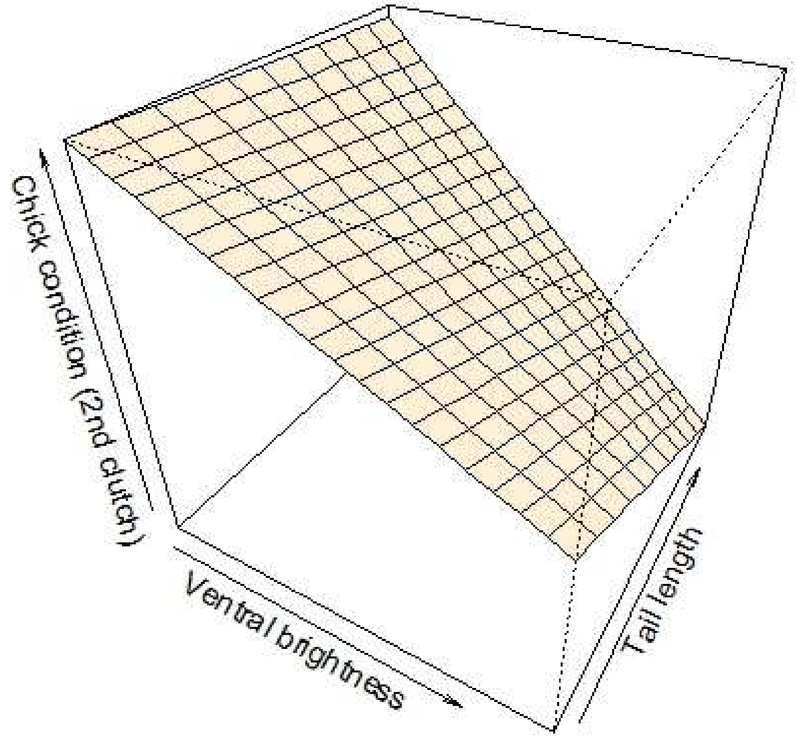

Figure 16: The effect of ventral brightness and tail length on chicks’ physical condition - 2^{nd} clutch (Model fit)

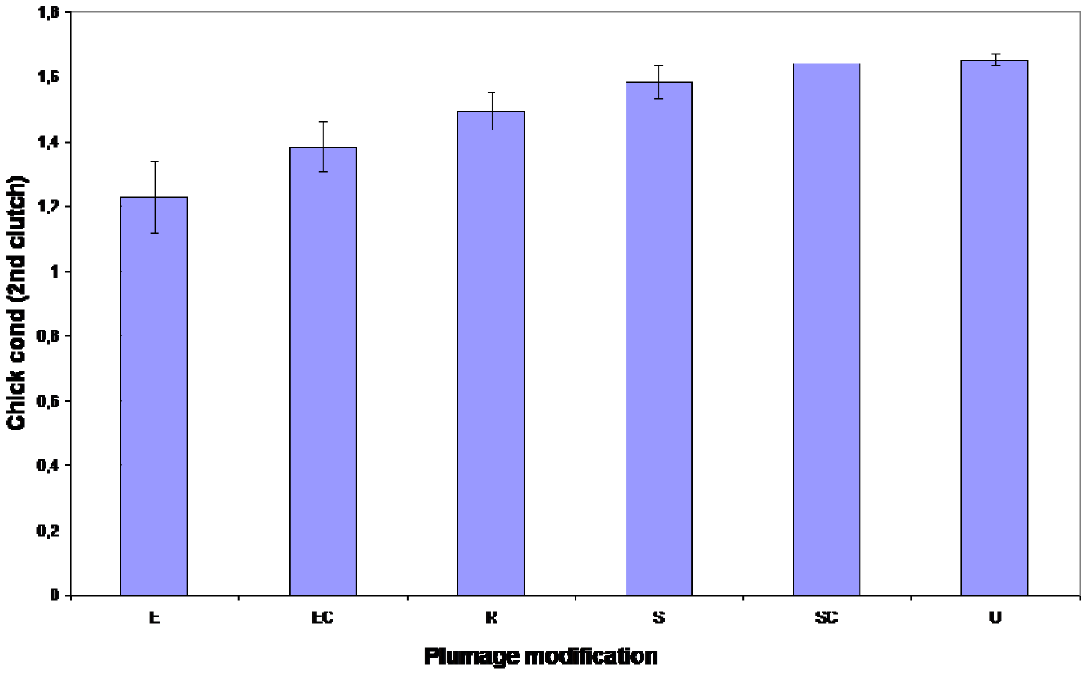

Figure 17: Effect of plumage manipulation on chicks' physical condition (2^{nd} clutch)

(For a description of levels of "plumage modification", see Table 2)

Chapter Five

Predator inspection activity in Trinidadian guppies (*Poecilia reticulata*): drab males are bolder than conspicuous males in the presence of females

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Abstract

Although females prefer to mate with conspicuously coloured males in numerous species, mate choice experiments in Trinidadian guppies (*Poecilia reticulata*) have shown that females prefer bolder males irrespective of their colouration when given the opportunity to observe their behaviour toward a potential fish predator. Given that coevolutionary systems are often subject to non-equilibrium dynamics, traits involved in the multi-component signalling system of guppies may not be expressed at the maximum possible level, which could lead to unreliable intersexual communication through the phenotypically plastic predator inspection behaviour. Here, we test experimentally the hypothesis that visually less conspicuous male guppies may compensate their lower quality-indicating value by being bolder than more conspicuous males in front of females through increased predator inspection activity, i.e. willingness to risk approaching predators during inspection without being killed. In the process we show that less visually conspicuous males, which invest less in one or more ornamental colouration traits, were often bolder than more conspicuous males for those traits. We go on to discuss the implications of this for the evolution of sexual signalling systems.

Introduction

According to communication theory, animal signals, and more specifically body colouration, are used by "signallers" to increase their own fitness by providing information to affect "receiver" behaviour (Johnstone, 1997; Endler, 2000; Dale, 2006). Choosing elaborately ornamented individuals as sexual partners can provide animals with direct and indirect benefits, information about which is transmitted by inter-individual differences in the degree of exaggeration of the ornaments (Pomiankowski, 1987; Andersson, 1994; Garamsziegi *et al.*, 2006; Bro-Jørgensen *et al.*, 2007). Models of sexual selection predict that these ornamental characters will be costly at evolutionary stability (Fisher, 1930; Zahavi, 1975; Iwasa *et al.*, 1991). This cost may be expressed during production and/or maintenance, and also through increased risk of predation or parasitism (Magnhagen, 1991; Folstad & Karter, 1992, Houde, 1997). Costly signals are efficient quality-indicators, as the costs they inflict to their bearers make them likely to be general viability indicators, in accordance with the handicap principle (Zahavi, 1975).

Despite the costs associated with production and reception of signals, animals often engage in multiple signalling systems. For example, the male wolf spider (*Schizocosa stridulans*) produces different components of its complex sexual display by using its abdomen and pedipalp simultaneously (Elias, 2006). Female crickets *Gryllus campestris* consider male call frequency as well as chirp rate to assess a mate, as both features signal different aspects of male quality (Scheuber *et al.*, 2004). And in the European barn swallow *Hirundo rustica rustica*, several ornaments related to reproductive success, such as tail streamer length, white spots in the tail and throat or ventral colouration, have been found to transmit information about quality of males and, perhaps, females (Møller, 1993, 1994; Kose *et al.*, 1999; Amundsen, 2000; Ninni *et al.*, 2004; Vaquero-Alba *et al*., unpublished data; **3**, **4**). Bro-Jørgensen (2009) argues that variation in selection pressures derived from fluctuations in ecological conditions and/or social context might lead to the emergence and maintenance of such multicomponent signalling systems.

Trinidadian guppies (*Poecilia reticulata*) provide an excellent example of multidimensional variation in sexually selected traits (Blows et al., 2003). They show complex colour patches based on carotenoids, pteridines and melanins (Houde, 1997) as well as several behavioural characters are involved in sexual selection. Thus, males exhibit a courtship display called the "sigmoid" display (Baerends et al, 1955; Liley, 1966; Houde, 1997), named that way because of the S-shape of the male's body when performing the display in front of the female. Another behavioural quality-indicating signal is "boldness", which is the willingness to risk approaching predators during inspection without being killed (Dugatkin & Godin, 1992; Wilson *et al*., 1994; Godin & Davies, 1995).

Several experimental studies on guppy populations have found evidence of a mating advantage for more conspicuous colour patterns in males (Haskins et al., 1961, Endler 1980, 1983). To avoid the confounding effects of predation, which may favour more cryptic patterns, Endler (1980) conducted a long term study in artificial streamers free from predators. He observed that males evolved increased conspicuousness in their colour patterns through a mismatch with the gravel background colouration. Carotenoid-related colours (red-orange-yellow) seemed to be the ones for which females showed a higher and more consistent preference. Female guppies preferred either males with bigger total body areas occupied by these pigments (Kodric-Brown, 1985; Houde, 1987; Brooks & Caithness, 1995a) or those with brighter orange spots (Kodric-Brown, 1989; Houde & Torio, 1992). Other studies have also tested other colour pattern characters, such as the area of structural-iridescent or black spots, but the results have not shown much consistency (see Houde, 1997) or vary among populations (Endler & Houde, 1995). Melanin-based black colouration in male guppies has been suggested to interact with orange colouration to determine mate choice, as a redundant signal (Brooks and Caithness, 1995a), or as an amplifier trait to improve detectability of orange spots (Brooks & Caithness, 1995b; Brooks, 1996). Another kind of quality-indicating secondary sexual character of males that could have an effect on female choice is the courtship display itself. Nicoletto (1993) found a positive correlation between the frequency of male displays and the frequency of female sexual responses. Stoner and Breden (1988) found that female guppies preferentially associated with the more frequently courting males, and Farr (1980) observed that males with higher rates of display obtained a disproportionately higher number of matings than males with lower rates of display.

Predation greatly influences different aspects of guppy biology. Expression of male colour patterns and behavioural characters such as sexual displays, as well as mate choice by females, seem to be determined to a great extent by the costs imposed by predation (Houde, 1997). Even sperm storage in females has been suggested to vary according to predation regime (Magurran, 2005). When they detect a predator, guppies exhibit inspection behaviour, which is thought to function as a way of recognizing and assessing the dangerousness of the predator (Magurran & Seghers, 1990; Dugatkin and Godin, 1992). Bolder males, i.e. those more willing to risk approaching predators during inspection without being killed, are better informed about predators near them, so they may have better chances of surviving encounters with them (Godin & Dugatkin, 1996). In a mate choice experiment in which male guppies were exposed to a cichlid fish predator, either live or a model of it, in the presence or absence of females, Godin and

Dugatkin (1996) demonstrated a positive correlation between the visual conspicuousness of the colour pattern of male guppies and their boldness toward the predator. Additionally, they found that although females prefer the most colourful males as mates, they show a stronger preference for bolder males irrespective of their colouration when they are given the opportunity to observe their inspection behaviour. Cichlid fish predators preferentially and consistently attack brightly coloured males rather than drabber ones (Godin & McDonough, 2003). Furthermore, being bolder toward a predator implies a willingness to incur greater costs in terms of predation risk and lost mating opportunities (Godin & Dugatkin, 1996). Thus, both characters have viability costs associated with them, which reinforces their reliability as indicator traits of male quality.

Predation regimes and other environmental and social factors are highly variable among different streams inhabited by guppies, which leads to an extreme degree of geographic variation in several characters (Endler, 1995; Houde, 1997). These differences result in dynamic selection pressures, which can explain the coexistence of multiple signals in guppy populations (Bro-Jørgensen, 2009). As coevolutionary systems are subject to non-equilibrium dynamics, traits in such systems may not be expressed at their maximum possible levels (see Saino *et al.*, 2003), leaving room for phenotypic plasticity in response to fluctuations in ecological and social environments. We hypothesize that in locations with lower risks of predation, less visually conspicuous males may be selected to increase their boldness at a low cost, in order to balance out their mating disadvantage due to lack of quality. This “compensation mechanism”, which has previously been proposed for barn swallows (Vaquero-Alba *et al.*, unpublished data; **3**, **4**), would help explain, at least in part, the high phenotypic variability observed in natural guppy populations. It would also contribute to understanding the persistence, in these populations, of males with lower colouration expression intensities, despite suffering a selective disadvantage due to their poorer performance during mate choice.

In the present study, we investigate the relationship between boldness and visual conspicuousness of male colour patterns of the Trinidadian guppy *Poecilia reticulata* during exposure to three model predators of varying dangerousness and in absence of predators. We predict higher boldness by less visually conspicuous males, as a way of compensating their lower intrinsic attractiveness to females, especially when exposed to models mimicking less dangerous predators when this compensation can be carried out at a lower cost.

Materials and methods

Trinidadian guppies are small fish belonging to the cyprinodont family Poeciliidae. They are native to streams and rivers of Trinidad and Tobago and adjacent parts of South America, they have internal fertilization and give birth to live young (Houde, 1997). Male and female guppies used in our experiment were first generation, laboratory-born descendants of wild guppies collected from the Aripo River, Trinidad, West Indies. All females were raised separately from males and were thus virgins when tested, as virgin female guppies are highly receptive to male courtship displays (Liley, 1968). Experimental males were allowed to court and mate with other females (not used in this study) in their home aquaria prior to being used in the current study. Fish holding aquaria for male guppies consisted of individual plastic tanks (20cm x 10cm, with a water level of 12 cm), each housing a single individual taken from a larger stock tank. Tanks contained a plastic plant and gravel substratum and were maintained at 23-25˚C under a 12 hour light / 12 hour dark illumination cycle. Females were kept in a large stock tank (100cm X 36cm, with a water level of 33cm) and under the same lighting and temperature conditions as males. Guppies were fed flake food in the morning, at least one hour before experiments began, and were given live brine shrimp nauplii in the evening. No food was available to the fish during experimental trials.

Male visual conspicuousness assessment

Fifteen bright and fifteen drab male guppies were selected based on the conspicuousness of their colouration. Each male was photographed on its left side using a Canon EOS digital SLR camera. Photographs were uploaded and analysed using UTHSCSA ImageTool software to calculate the areas of different colouration patches: orange, yellow, black, white-silver, blue-violet and bronze-green, on the body of each male. The total area occupied by all the colouration patches on the left side was calculated as a proportion of the total left-side surface area. This proportion was then used to categorize males into “conspicuous” and “drab”. Males with a proportion of less than 20% of their total left-side surface area occupied by colouration patches were allocated as drab, and males of 20% and above were considered as conspicuous. The highest recorded colouration cover was 34.79% and the lowest was 10%. Once being categorized as either visually “conspicuous” or visually “drab”, males were assigned into pairs with the most conspicuous males being paired with the least drab males, in order to keep the percentage difference as constant as possible. The average difference in conspicuousness was 14.01%.

Experimental trials

Experimental trials were carried out in a tank 120 cm long and 36 cm wide, with a water level of 15 cm and gravel substratum. It was divided into 3 compartments: a small compartment (15cm in length) that housed the predator model, separated from the rest of the tank by a permanent clear Perspex partition, allowing the model predator to be visible to both male and female guppies throughout experiments. Another small compartment (30 cm in length) at the opposite end of the tank contained the two male guppies during the acclimation period; throughout this time an opaque partition blocked their view from the predator compartment. Two virgin female guppies were present in each experiment and were separated from males in a clear plastic cylinder (10.5 cm in diameter, 32.97 cm in circumference). The cylinder was perforated, so both males and females were able to respond to visual and chemical cues. Females were restricted from free-swimming in the test tank to minimise distraction of the males and to prevent sneak mating attempts. The large section between the predator and acclimatisation compartments was further divided into the risky zone, and safe zones 1 and 2. The risky zone was measured as 15cm from the edge of the predator compartment (approximately 6 guppy body lengths); safe zone 1 was the area between the end of the risk zone and the female guppy cylinder (40cm); safe zone 2 was the area between the female guppy cylinder and the opaque partition (20cm), the farthest from the predator compartment and therefore the least "risky" zone. The risky zone was further divided into 6 smaller zones, according to their risk level due to their proximity to the predator chamber. Three model predators of varying perceived dangerousness were used in our experiment: the high-intensity predator was represented by a model of the pike cichlid fish *Crenicichla alta*, the medium-intensity predator by a model of the blue acara fish *Aequidens pulcher*, and the low-intensity one by a model of the rivulus fish *Rivulus hartii*. All these species are all known natural guppy predators in the Aripo River region (Reznick & Endler, 1982; Reznick *et al.*, 2001). Before each trial, a pair of males was put in the compartment at the opposite end of the predator compartment, and males were allowed to acclimatise for a period of 30 minutes. Following this period, the opaque partition was raised slowly, allowing the male guppies to view the females in the cylinder and the predator compartment that contained the model predator. Trials began as soon as both males left the acclimatisation zone and lasted for 30 minutes. Once in the main test chamber, each individual male guppy had the choice of either initiating an inspection visit (the riskiest option), following the other male who initiated the approach, or not inspecting at all. Fifteen pairs of males were similarly tested for each of the three aforementioned predator treatments separately. Different females were used for each trial. Therefore, individual fish were only used once in each experiment. Each trial was recorded using a small video camera attached to a tripod and visual observations were made from behind a white blind through a small opening, to minimize any behavioural disruption. Males were randomly allocated to different predator treatments. The order in which males were allocated to different model predators, as well as the time at which each trial was carried out were recorded.

Shyness score (named that way because its value increased with increasing distance from the predator) was calculated by allocating each male a score every 30 seconds depending on his position in the test chamber: they were allocated a score of 15 in safe zone 2, a score of 10 in safe zone 1, and a score of 1 (closer to the predator) to 6 (farther from the predator) in risky zone. Therefore, bolder males acquired lower shyness scores. *Time in risky zone* was calculated as the amount of time each male spent in the risky zone in 30 minutes. *Number of first inspections* was calculated as the total number of predators inspections (swimming from safe zones to the risky zone) initiated and led by each male during each trial.

Statistical analyses

Multicollinearity is an interdependency condition that can be observed in a multiple regression in which several linear relationships hold between variables. It can exist independent from any relationships between the response variable and the explanatory variables, and may make the coefficient estimates change erratically in response to small changes in the model or the data (Frisch, 1934; Farrar & Grauber, 1967). As several of our explanatory variables and interactions were related to areas of different body colouration patches of male guppies, we checked them for multicollinearity, using the *vif.mer* function of *mer-utils.R* online repository (Frank, 2011). Parameters with values of *vif.mer* under 5 are considered to suffer from acceptably levels of multicollinearity.

We analysed the variables influencing several indicators of boldness in male guppies, using generalized linear mixed effects models (GLMM; details explained below) implemented with the *lmer* function of *lme4* package (Bates & Sarkar, 2007) of R 2.13.0 (Crawley, 2007; R Development Core Team, 2010). Using GLMMs, it is possible to analyse the influences of both random and fixed effects on response variables (Calabuig *et al*., 2010).

Factors affecting shyness score and time in risky zone were analysed using a Gaussian error structure and an identity link function. Factors affecting the number of first inspections were analysed using a Poisson error structure and a log link function.

For all the analyses, body length and areas of carotenoid-based (orange+yellow), black, and structural (white-silver+blue-violet+bronze-green) colouration patches, were included as covariates, and predator type was included as a fixed factor. “Pair identity” was included as a random term in our models. In doing so, we controlled for the nonindependence of the data points that occurred because individuals within a pair could have influenced each others' behaviour. Also, time when each trial took place and order of allocation of pairs to different model predator treatments were included as random effects, to control for the potential confounding effects of these variables.

GLMMs were initially fitted with all explanatory terms included in them, including first-order interactions in which body length and/or colouration ornaments were involved. A summary of the maximal models fitted is given in Table 1. Minimal adequate models were chosen after a process of stepwise deletion of the least significant effects ($p>0.05$), starting with the highest-order interactions, providing the simplification did not significantly reduce the explanatory power of the model (Crawley, 2002). Consecutive models were compared using chi-square (χ^2) statistics. The residuals from the models were checked for normality and homocedasticity; where appropriate, transformations of the response variables or standardizations of the explanatory variables were done following R Development Core Team (2010).

Results

After standardizing the explanatory variables of our models, carotenoid colouration area and structural colouration area yielded *vif.mer* values slightly higher than 5, although still several orders of magnitude lower than the same variables before standardization. The rest of the single variables and 2-way interactions included in the analyses had *vif.mer* values under 5, and most of the times under 4.

Our analyses showed a significant 2-way interaction between body length and carotenoid-related colouration area on shyness score ($n=90$, $\chi^2_1=5.549$, $p=0.018$; Table 1). Male guppies with smaller areas of carotenoid-based colouration showed lower shyness scores than those with bigger areas. Amongst males with bigger carotenoid colouration areas, smaller males had lower shyness scores than those with a longer body

(Figure 1). There was also a significant 2-way interaction between area of carotenoid-based colouration and black coloured area on shyness score (n=90, χ^2_1=4.855, p=0.030; Table 1). Males with both bigger carotenoid-based colouration areas and bigger black colouration spots showed lower shyness scores than the rest. Amongst males with smaller black colouration patches, those with bigger carotenoid-based colouration areas had slightly lower shyness scores than the ones with smaller carotenoid colouration areas (Figure 2).

When analysing the number of first inspections by males, we saw a significant effect of male black colouration area on the response variable (n=90, χ^2_1=8.655, p=0.003; Table 1). Male guppies with smaller black colouration areas initiated significantly more predator inspections than those with bigger areas (Figure 3). There was a significant effect of male body length on the number of predator inspections initiated and led by males (n=90, χ^2_1=19.562, p<0.0001; Table 1). Bigger males initiated slightly more predator inspections than smaller sized males, being this effect significant (Figure 4). We observed a significant covariation between the size of carotenoid-based colouration area and the number of first predator inspections (n=90, χ^2_1=34.233, p<0.0001; Table 1). Males with smaller carotenoid colouration areas initiated significantly more predator inspections than those with bigger areas. (Figure 5).

Our analyses also revealed a significant effect of melanin-related (black) colouration area size on time spent in the risky zone (n=90, χ^2_1=5.328, p=0.021; Table 1). Male guppies with smaller areas of black colouration spent significantly more time in the risky area than those with bigger black areas (Figure 6).

We did not detect any effect of predator type on either shyness score (n=90, χ^2_3=0.101, p=0.951; Table 1), number of first inspections (n=90, χ^2_3=1.006, p=0.605; Table 1) or time spent in risky zone (n=90, χ^2_3=0.790, p=0.674; Table 1).

Discussion

The results of our study suggest the existence, at least to a certain extent, of a compensatory strategy through predator inspection in males with poorer indicator traits, as we predicted. Male guppies with smaller areas of black colouration spent more time in the risky zone, closer to the predator chamber, and males with smaller body size or smaller areas of carotenoid-related colouration got lower shyness scores, than bigger and/or more coloured males. Lower shyness scores and longer times in risky zone reflect bolder males. Also, males with either smaller areas of carotenoid-based colouration, black colouration, or both, initiated and led a much larger number of predator inspections than those with bigger areas. All this is indicative of a higher boldness, and a willingness to incur greater costs, in terms of predation risk and lost mating opportunities (Godin & Dugatkin, 1996). Male guppies with more carotenoid colouration, as well as more of other types of colour, are preferred by females as sexual partners (Kodric-Brown, 1985; Houde, 1997). Likewise, females prefer bigger males in certain populations (Reynolds & Gross, 1992). Thus, male guppies with a lower intensity of expression for one or more of these characters should be selected to increase their chances of being chosen by females by increasing their predator inspection activity. This trend somehow reversed when analysing specific interactions. Males with both bigger areas of carotenoid-related and black colouration showed lower shyness scores, whereas bigger-sized males initiated more predator inspections . According to these results, males with a higher total investment in simultaneously exaggerating certain, expensive sexual traits, may still afford to show a bolder behaviour in front of females, suggesting a higher individual quality. When considering these sexual signals separately or in different interactions, however, there seems to be a trade-off between the resources allocated to exaggerating the expression of ornamental traits and those left for engaging in a bolder behaviour. Alternatively, and more plausibly, the costs of the alternative ways of signalling may vary, allowing only males with less costly combinations of simultaneously exaggerated traits to engage also in predator inspection. A greater conspicuousness means greater conspicuousness to predators (Godin & McDonough, 2003), and good physical condition is required to successfully escape from predator attacks. Thus, only lower-quality males that did not make a heavy investment in one or more colouration-related traits may be selected to maximise the phenotypically plastic predator inspection behaviour, in an attempt to increase their chances of being chosen by females and maximise their reproductive success.

Our study complements that of Godin & Dugatkin (1996), and reinforces the importance of predator inspector behaviour as a sexual signal. They concluded that visual conspicuousness of the colour pattern of males correlates positively with boldness toward a cichlid fish predator. Our results, however, show that this is not necessarily always the case, and boldness can actually serve as a compensatory strategy for less visually attractive males, as well as a redundant sexual signal for more attractive ones. They also highlight the geographic variability in the expression of different traits used by guppies for signalling purposes (Endler, 1995; Houde, 1997), as the fish used by Godin & Dugatkin came from wild guppies collected from Quaré River, rather than Aripo River, from which the parental generation of the ones we used came.

Several authors have reported that more conspicuously coloured males are higher quality (Endler, 1980; Nicoletto, 1991, 1993). If this was always the case, we would expect more conspicuous males to signal through predator inspection at a higher rate than less conspicuous ones. However, in our study, this trend was only found for specific interactions between colouration traits, whereas most of the results suggest higher predator inspection activity by less conspicuously coloured males. Female choice seems to be based on multiple criteria, in guppies (Kodric-Brown, 1993) and many other species (e.g. Kodric-Brown, 1990; Møller, 1994; Elias *et al*., 2006; Vaquero-Alba *et al.*, unpublished data; **3**, **4**). The relative importance of each of the traits involved in sexual signalling changes with social and environmental factors, and is determined by the behavioural context (Kodric-Brown, 1993). Contrary to the static point of view of earlier research, the latest studies on multiple-signalling species are helping us understand how multi-component signalling systems, including both redundant or non-redundant signals, can be explained by environmental fluctuations (including both ecological conditions and social contexts; Bro-Jørgensen, 2009). Non-equilibrium dynamics to which signaller-receiver systems are often subject can lead to unreliable communication, at least when considering the quality-indicating value of certain ornamental colouration signals alone, and highlight the importance of more condition-dependent, phenotypically plastic traits (like carotenoid-related colouration and predator inspection behaviour) for coping with dynamic selection pressures. Thus, only higher quality males may be able to maximise both visual conspicuousness and boldness, and counter the predation costs associated with their greater attractiveness to predators, provided they are better at escaping from predators due to their better physical condition (Godin & Dugatkin, 1996). Considering ornamental traits alone or in particular trait combinations, however, only males with lower investment levels in one or more of those traits may show an enhanced boldness. This is actually what we saw in our study.

Contrary to our initial prediction, predator type did not have any effect on predator inspection behaviour, and males were not bolder or more timid in front of predators with varying levels of dangerousness. As the males we used for our experiment had never experienced a real predator before, the only behavioural differences we should expect are those with a genetic basis, shaped by natural selection, rather than those due to learning and experience. Furthermore, as we used model predators instead of real ones, male guppies did not have the possibility of experiencing different behaviours from the different predators themselves, and react accordingly. Further experimentation with live predators is required, to test whether there is actually a different response of male guppies, in terms of boldness, towards predators with different levels of dangerousness.

To sum up, only male guppies with a high degree of exaggeration in the expression of certain traits or combinations of traits (like body size or carotenoid-based and melanin-based colouration areas) could afford to show also a high degree of boldness through an increased predator inspection activity, presumably due to their higher quality and behavioural vigour. However, when analysing quality-indicating traits alone or in other trait combinations only less visually conspicuous males with lower investments in one or more colouration-related, ornamental traits, seemed to be able and/or willing to compensate their lower attractiveness to females by increasing their levels of predator inspection.

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Table 1 Summary of minimal adequate models

Response variable	N	ΔAIC	Explanatory variable	χ^2(df)	p
Shyness score[†]	90	22	Body length	2.483 (1)	0.115
			Carotenoid	0.1367 (1)	0.712
			Black	1.917 (1)	0.166
			Predator	0.101 (2)	0.9507
			Body length:carotenoid	5.549 (1)	0.018
			Carotenoid:black	4.855 (1)	0.030
Number of first inspections[†]	90	14.8	Body length	19.562 (1)	<0.0001
			Carotenoid	34.233 (1)	<0.0001
			Black	8.655 (1)	0.003
			Predator	1.006 (2)	0.605
Time in risky zone[†][‡]	90	18.7	Black	5.328 (1)	0.021
			Predator	0.790 (2)	0.674

ΔAIC difference between AIC of maximal and minimal model, *black* area of melanin colouration (black), covariate, *body length*, covariate, *carotenoid* area of carotenoid colouration (orange+yellow), covariate, *pair id* pair identity, factor with 15 levels*, predator* predator treatment, factor with 3 levels, *structural* area of structural colouration (white-silver+blue-violet+bronze-green), covariate, *timeday* time of day, factor with 58 levels, *trial order* order of allocation of pairs to predator treatments, factor with 6 levels.

Fixed effects and covariates included in maximal models: body length, carotenoid, black, structural, predator, body length:carotenoid, body length:black, body length:structural, body length:predator, carotenoid:predator, black:predator, structural:predator, carotenoid:black, carotenoid:struct, black:struct, random effects: pair id, timeday, trial order.

† Explanatory variables were standardized: stand(x)=x-mean(x))/sd(x)
‡ Response variable was tangent transformed

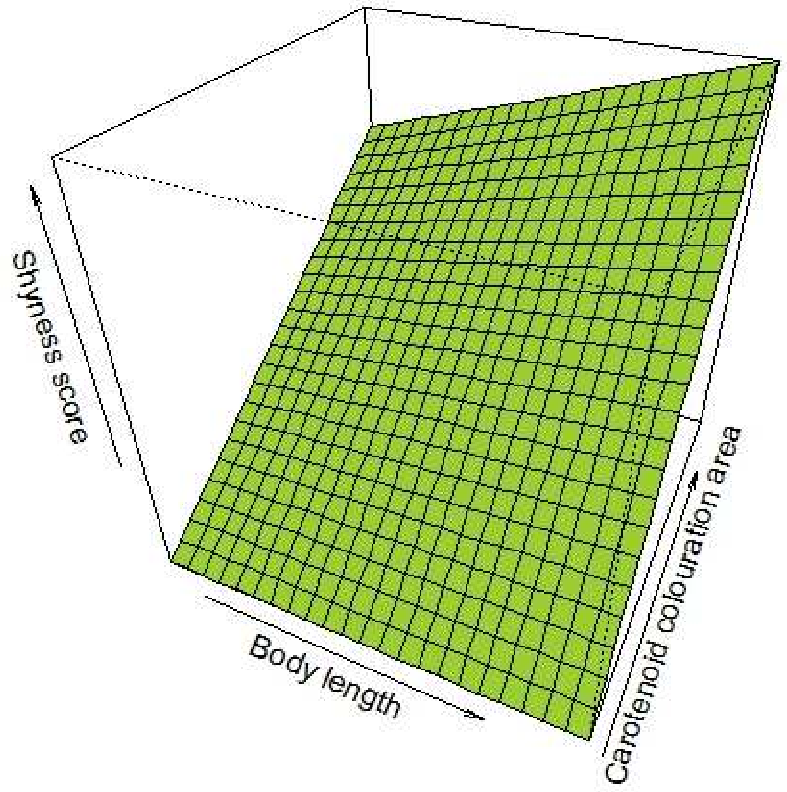

Figure 1: The effect of body length and carotenoid-based colouration area on shyness score (model fit)

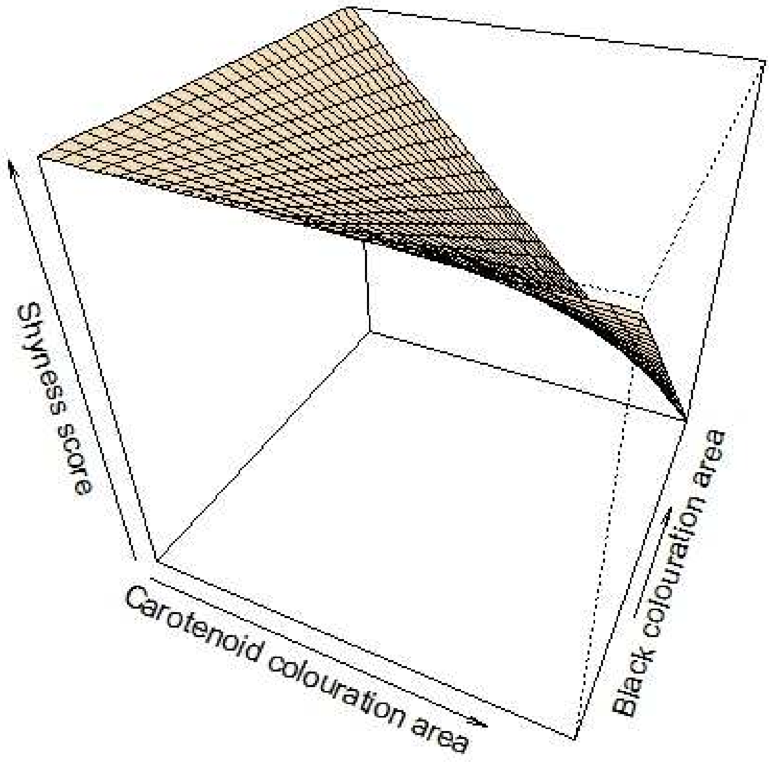

Figure 2: The effect of black colouration area and carotenoid colouration area on shyness score (model fit)

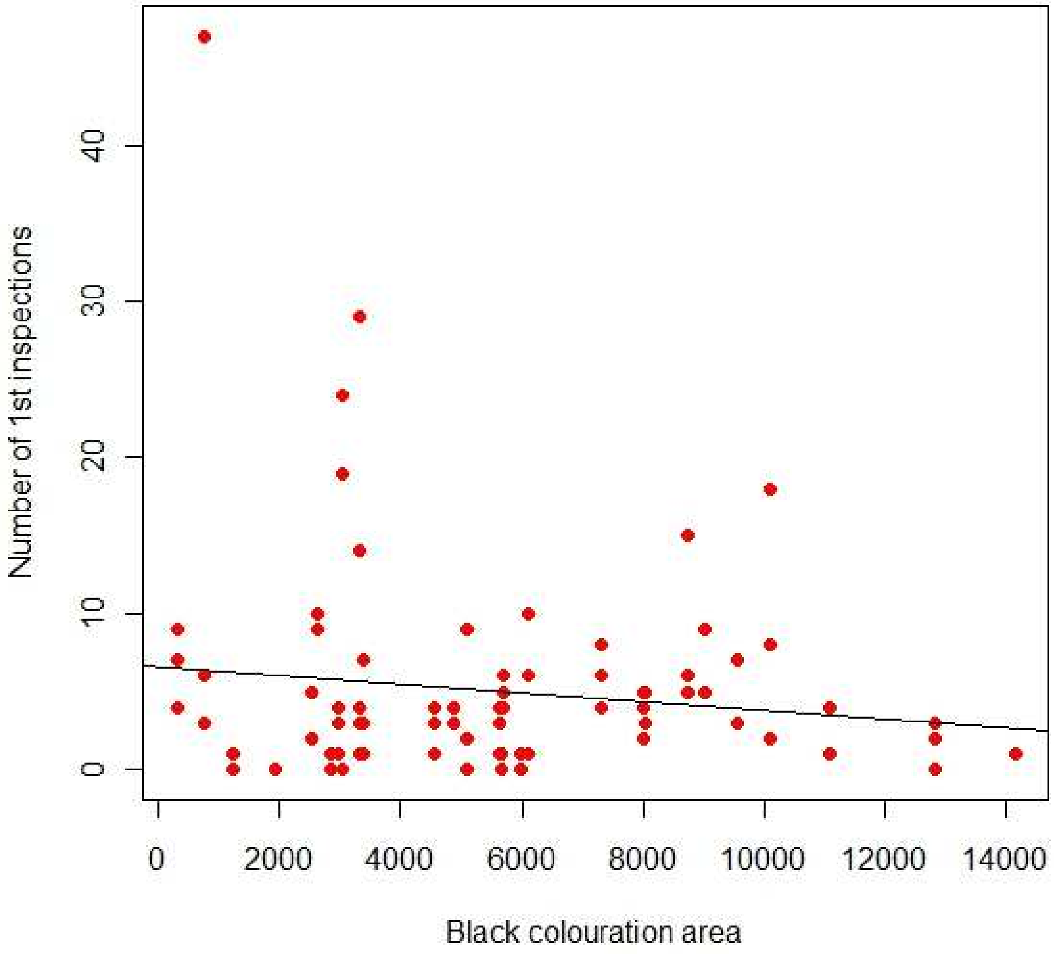

Figure 3: Effect of male black colouration area on

number of first inspections

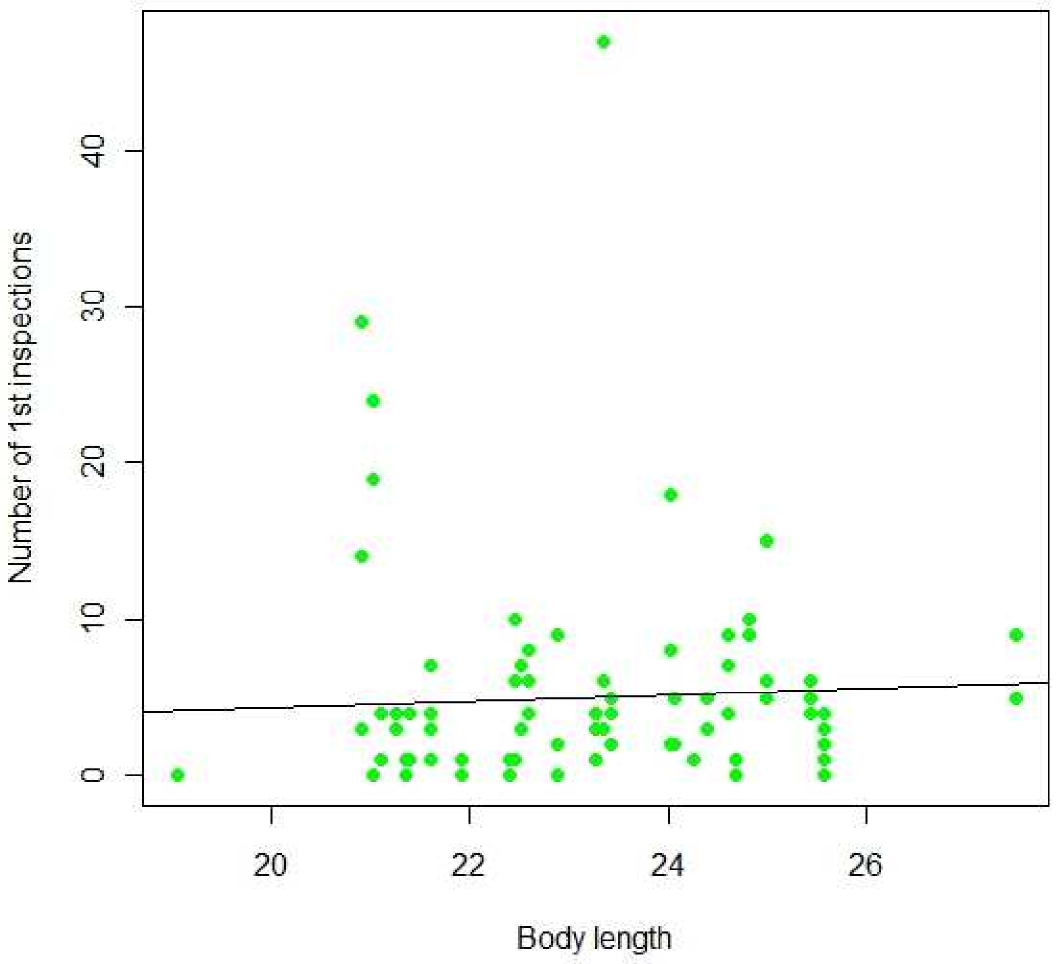

Figure 4: Effect of male body length on

number of first inspections

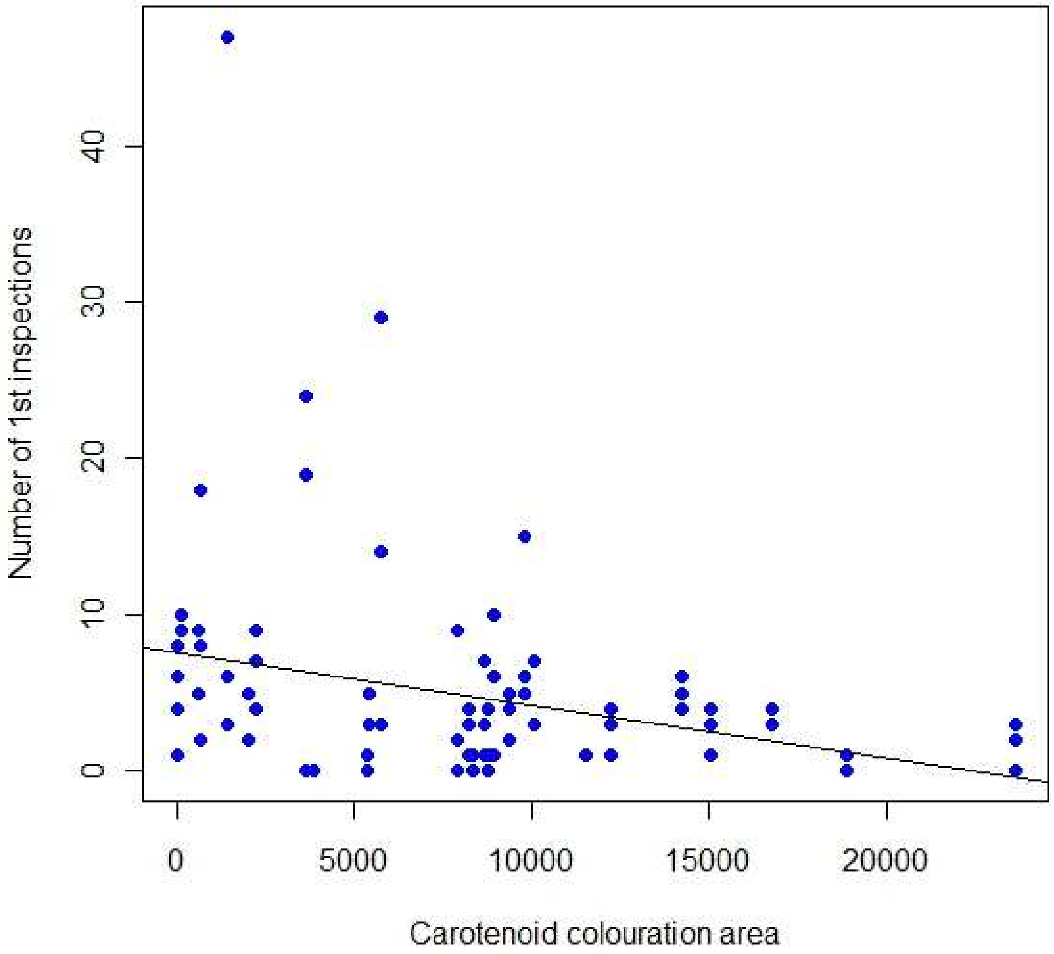

Figure 5: Effect of male carotenoid colouration area on

number of first inspections

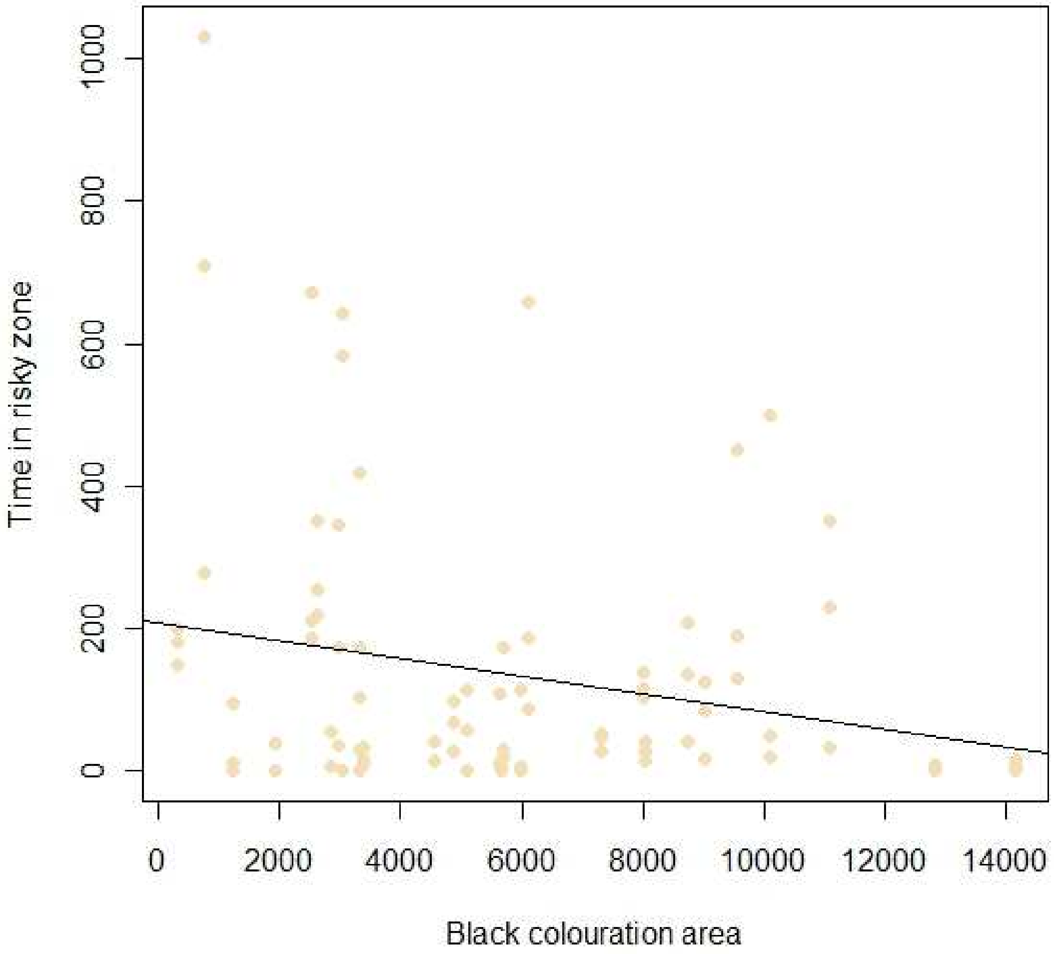

Figure 6: Effect of male black colouration area
on time spent in risky zone

Chapter Six

General discussion

In the introduction (**1**), the conditions that might lead to the emergence and maintenance of multicomponent signalling systems in animals were reviewed, and the evolutionary consequences of dynamic variation in selection pressures and/or non-equilibrium dynamics in sexually antagonistic coevolution were discussed. Through the testing of the so-called "compensation mechanism" hypothesis, the experimental work presented in this thesis explores the possibility of multicomponent sexual signalling systems used by animals being selected to optimise rather than maximise the amount of information they transmit in relation to the costs they incur.

The objective colour assessment conducted in the field and in the lab and the repeatability calculations for each technique separately and between the techniques indicated a higher reliability for the lab option, consisting in collecting feather samples from live birds and assessing their colouration in the lab, after arranging them in a way that mimics the original plumage pattern. As this technique considerably reduces the handling time of the birds, minimising the stress levels inflicted on them, as well as being easier on equipment, it should be the common way of assessing colour objectively and doing repeatability calculations.

We demonstrated the value of the novel GLMM-based method for repeatability calculation (Nakagawa & Schielzeth, 2010). Thanks to the possibility of accounting for random factors, it allows to calculate adjusted repeatabilities, more accurate than those calculated with other methods, e.g. ANOVA-based, increasing the power of the tests.

Pigment-based plumage markings as quality indicators

Using European barn swallows as field models of multicomponent signalling species, we investigated the effect that several ornamental traits potentially used with sexual signalling purposes exert on a number of well-known reproductive success indicators (Vaquero-Alba *et al.*, unpublished data; **3**, **4**). The ornamental traits studied included ventral plumage colouration, a character largely overlooked in previous sexual selection studies for this subspecies, and a specific consideration was given to interactions between the variables studied. The results of our study suggest that throat and ventral colouration patches, as well as tail streamer length, may play an important role in the sexual signalling system of the European barn swallow. Swallows with higher investment in darker plumage patches, either alone or in interaction with tail streamer length or physical condition, performed generally better according to the values of direct indicators of reproductive success (Vaquero-Alba *et al.*, unpublished data; **4**), and females mated to darker-throated males laid significantly sooner during the breeding season, an indirect indicator of fitness (Vaquero-Alba et al., unpublished data; **3**). Remarkably enough, elongated tail streamers seemed to have quite a weak effect in predicting reproductive success in our population compared to pigment-based plumage markings, and contrary to previous similar studies in the European barn swallow (reviewed in Møller, 1994; Turner, 2006). Actually, tail streamer length did not predict patterns of assortative mating nor laying date, although it determined chick condition in second clutch to a great extent. A possible explanation could be that the barn swallows in the population we studied have suffered an evolutionary process different from the other European populations studied, as a consequence of the adaptation to local ecological and/or social conditions. For example, intensification of farming practices along the continent, with livestock numbers decreasing and hedgerows and waterbodies being scarcer than in the past, may be associated with reduced invertebrate numbers (Evans, 2001; Møller, 2001, Vickery *et al.*, 2001). Such changes in ecological conditions may differentially affect barn swallow populations, according to the extent of the habitat manipulation. However, whether this is having an influence in the sexually selected signals used by this species, it may be too soon to make inferences about it, given that the biggest changes in farming activity have been happening essentially for the last few decades. In any case, the findings of our study indicate that dynamic variation in selection pressures (Bro-Jørgensen, 2009) may be shaping the evolution of sexually selected multiple signals in the European barn swallows, perhaps leading to speciation.

It may be difficult to understand how female choice can evolve in species with no apparent direct benefits of choice to females, as it seems to happen in the species we studied. The most extreme case is lekking species, where in spite of males giving no resources to females or parental care to offspring, female preference has evolved and maintained. A possible explanation could be that females receive “good genes” for their offspring from preferred males. But under this assumption, we would expect genetic variance to be depleted by directional selection induced by female choice, as the lek paradox states (Taylor & Williams, 1982; Andersson, 1994; Rowe & Houle, 1996), and multicomponent signalling systems would hardly ever evolve. Rowe and Houle (1996) argue that sexually selected traits may favour genetic variance, given two premises: expression in sexually selected traits being condition dependent, and condition having high genetic variance. Both of which are well supported (see Houle, 1991; Andersson, 1994). This would help resolve the lek paradox. Alternatively, Pomiankowski & Møller (1995), suggest that under directional selection, greater additive genetic variance can be maintained if exaggeration of sexually selected traits increases fitness at a greater than linear rate. Through their model, they invoke what they call “modifier loci”, arguing that directional selection increases the number of loci that affect the sexually selected trait or increases each locus’ contribution to it. Under certain circumstances, our “compensation mechanism”, largely based on Bro-Jørgensen’s “fluctuating environments” hypothesis (2009) and van Dorn’s “sexually antagonistic coevolution” one (2006) could also explain how multiple signals could emerge. Our hypothesis works especially well, we think, for the case of monogamous species, where the number of available mates decreases with time, and obviously, the quality of the remaining, unchosen ones. Lower-quality individuals could be selected to develop a compensation strategy through phenotypically plastic traits as, at a certain point, that could make them become the most exaggeratedly-signalling ones (when only lower-quality individuals remain available).

Tail streamer manipulations affected reproductive success indicators quite much in the predicted direction. Ventral colour manipulation, however, did not have a remarkable effect on hatching success or chick condition (Vaquero-Alba et al., unpublished data; **4)**. Nevertheless, females mated to males with simultaneously artificially shortened tails and enhanced ventral colouration seemed to “penalize” their partners’ lack of “honesty” by laying smaller clutches. If this behaviour was due to these males having more parasites than expected from their external appearance, it could be argued that ventral colouration functions as an honest signal of quality through susceptibility to parasite infestation (see Fitze & Richner, 2002), but more consistent results are needed. Tail streamer manipulation has been extensively applied to the European barn swallow during the last couple of decades (e.g. Møller et al., 1995, Evans, 1998) and the technique is well developed and tested. It’s the first time to our knowledge, however, that ventral colour manipulation technique is applied to this subspecies, although it has been used in the North American barn swallow, a different subspecies, before (e.g. Safran et al., 2005). Thus, the method may still need to be "finely tuned" to the particular idiosyncrasy of the European subspecies, so that more consistence evidence is gathered for quality-indicating value and female preference for darker plumage colouration, as other evidences gathered during our investigation suggest (Vaquero-Alba et al., unpublished data; **4**).

Compensating through habitat quality and predator inspection

The findings of our investigations support the hypothesised "compensation mechanism", at least to a considerable extent: barn swallows investing more heavily in "cheaper" ornaments (pigment-based plumage markings), or, for certain combinations of traits, showing some kind of "weakness" either in their sexual displaying intensity or physical condition, seem to be selected to defend breeding sites associated with higher-quality foraging areas (Vaquero-Alba *et al.*, unpublished data; **3**). Likewise, brighter individuals breeding in sites next to higher quality areas achieved higher values for several reproductive success indicators (Vaquero-Alba et al., unpublished data; **4**). The fact that other equally bright individuals bred in sites surrounded by poorer quality areas and achieved a lower reproductive success may be indicative of differences in individual quality amongst brighter-coloured swallows. If animals are selected to optimise rather than maximise the information content of sexually transmitted signals (**1**), we may expect some swallows not to express their plumage colouration patches at the maximum possible level, perhaps because they are saving themselves for future breeding attempts. These individuals might be selected to put a higher premium on foraging and territorial aggression, compensating to a certain extent their lack of signalled quality at a lower cost than using morphological characters, and keeping themselves in good condition.

Using Trinidadian guppies as lab models of multicomponent signalling species, we investigated the effect that several ornamental traits potentially used with sexual signalling purposes exert on various indicators of “boldness”, i.e. the willingness to risk approaching predators during inspection without being killed (Dugatkin & Godin, 1992; Wilson *et al*., 1994; Godin & Davies, 1995). Although previous studies had shown a bolder behaviour for more conspicuously ornamented males (Godin & Dugatkin, 1996), we present evidence that this is not necessarily always the case, and boldness can serve as a compensatory strategy for less visually attractive males, as well as a redundant sexual signal for more attractive ones (Vaquero-Alba *et al*., unpublished data; **5**). Male guppies with a lower intensity of expression for one or more quality-indicating traits, or with exaggerated combinations of traits which are not very costly to express, should be selected to increase their chances of being chosen by females at a lower cost, by increasing their predator inspection activity. As for the case of the barn swallows, lower intensity in the display of certain ornamental traits in guppies could be due to those traits being costly to express, or to certain individuals decreasing their investment in signal exaggeration in order to save themselves for future mating attempts. These individuals might be selected to engage in a bolder behaviour, which would be a “cheap” way of compensating for the lack of displayed quality while keeping themselves in good condition for the future.

The study of multicomponent signalling systems: future prospects

Future repeatability calculations need to be performed using the GLMM-based method developed by Nakagawa & Schieltzeth (2010), in order to improve their accuracy thanks to increased test power. Comparability between field and lab-based objective colour assessment techniques can be carried out for other bird species, especially those with other types of colouration, to consistently assess the reliability of the more convenient lab technique for objective colour assessment (Vaquero-Alba et al., unpublished data; **2**).

Further work is needed to gather more empirical evidence for the "fluctuating environments" hypothesis (Bro-Jørgensen, 2009), the "sexually antagonistic coevolution" hypothesis (van Doorn & Weissing, 2006), and the "compensation mechanism" hypothesis(**1**; Vaquero-Alba *et al.*, unpublished data; **3,4,5**). Likewise, hypotheses that have tried to unravel the lek paradox (Rowe & Houle, 1991; Pomiankowski & Møller, 1995) need to be theoretically and empirically tested to increase the amount of evidence supporting them, and see how our predictions can fit into the new ideas about multiple signalling. Using the powerful computing capacity of the new GLMM based statistical tools, new analyses on sexually selected multicomponent signalling systems can be performed with a specific focus on the interactions between the traits studied (e.g. Garamszegi, 2006, Vaquero-Alba *et al.*, unpublished data; **3,4,5**), including multiple component systems which have been usually regarded as single traits (Scheuber, 2004; Elias *et al.*, 2006). Additionally, existing work can be critically reviewed to find evidence for multiple cues providing additional information or serving as back-up signals, and sexual conflict favouring the evolution of multiple signalling (Candolin, 2003; Grether *et al.*, 2004). Non-equilibrium scenarios, triggered by sexual conflicts and favouring the coexistence of multiple sexual signals, need to be investigated in more depth (Holland & Rice, 1998; Arnqvist & Rowe, 2005), so that we can improve our understanding of the complex processes leading to the emergence and permanence of multiple signalling systems, as well as the intercorrelated selective pressures to which traits being part of these systems are subject and the interactions which arise between them.

We hope our work adds some interesting ideas and predictions to a topic which is currently under intense controversy, and suggests some interesting directions which future investigation could take, in order to promote a productive debate in an area of knowledge that we find fascinating and intellectually challenging.

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