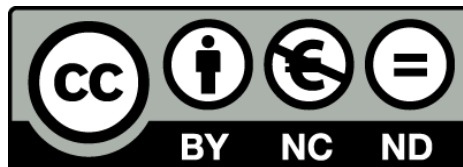# The Colours of Choice: Multiple signalling through feather colouration

Ornamentos múltiples: Mecanismos, función y evolución

Fernando Mateos González

The Colours of Choice

Multiple signalling through feather colouration.

Ornamentos múltiples: Mecanismos, función y evolución.

Fernando Mateos González
PhD Thesis 2012

The Colours of Choice: Multiple signalling through plumage colouration.

Ornamentos múltiples: Mecanismos, función y evolución.

Programa de doctorat: Biodiversitat

Memoria de tesis presentada por Fernando Mateos González para optar al título de Doctor.

El Doctorando

Vo Bo del Director y Tutor de tesis:

Dr. Joan Carles Senar Jordá
Director de Tesis

Dr. José Domingo Rodríguez Teijeiro
Tutor de tesis

Contents

Richard P. Feynman

"You can know the name of a bird in all the languages of the world, but when you're finished, you'll know absolutely nothing whatever about the bird... So let's look at the bird and see what it's doing. That's what counts."

Introduction

In sexual or social selection processes, animals employ traits that signal qualities. The use of multiple signals is known to be well spread in numerous species, but the ultimate reasons for using more than one single signal are not easy to explain. Why would animals invest in several different signals, instead of focusing on only one?

The numerous hypotheses proposed to explain the existence and function of multiple signals (reviewed in Candolin 2003) can be classified in adaptive, non-adaptive and maladaptive hypotheses. The first group includes those hypotheses that consider multiple signals as adaptive traits that increase the individual's fitness by reducing choice errors or costs. Given their evolutionary significance, these hypotheses have triggered substantial interest, especially the one known as *Multiple Messages Hypothesis* (Møller & Pomiankowski 1993). It states that different signals provide information about different qualities, that can be evaluated together or individually by different receivers.

Traditionally, these adaptive hypotheses have been framed into static scenarios, under constant selection pressures (Bro-Jørgensen 2010). However, there is increasing awareness of the need to widen this static framework, by taking into account the selection plasticity produced by spatial and temporal environmental heterogeneity (Cornwallis & Uller 2010).

A recent review suggests that fluctuating ecological and social environments could also have an important role in the origin and maintenance of multiple signals (Bro-Jørgensen 2010). In an environment where changes happen faster every day, multiple signalling would allow a more flexible, dynamic selection, increasing the odds of survival and fitness (Bro-Jørgensen 2010). The evolutionary implications of this hypothesis highlight the importance of novel studies in multiple signalling.

The role of bird colouration in multiple signalling

Arguably, the most remarkable feature of birds is the striking diversity of colourations they show on their plumage. This conspicuous attribute has lured scientists to try to understand the adaptive value of colouration since the early ideas of Darwin and Wallace. Wallace was a resolute defender of natural selection as the motor of colour diversity in birds (Wallace 1889). Darwin (1871), however, relied on sexual selection to explain the evolution of male bright colours.

Both of them have been proven right. While there are merely a few cases in which colour traits could be explained as the outcome of gene pleiotropy only (Buckley 1987; Cooke & Buckley 1987), a wide array of functions support Wallace's view (Bortolotti 2006). For example, feather colours have been proposed to protect from abrasion (Burtt 1986) and UV radiation (Burtt 1979) and to aid thermoregulation (Wolf & Walsberg 2000; Negro et al. 2006). In addition, dark coloured structures across or around the eyes have been suggested to enhance vision, reduce reflections and glare, or to hide the eyes (Cott 1940; Gavish & Gavish 1981; Burtt 1984; Wheeler 2003). But, probably, the most fruitful research field on naturally selected functions of plumage colouration has been the one regarding the relationships between prey and predator. Predators and preys keep evolving a rich display of mechanisms of concealment, advertisement and disguise, in a never ending escalating race for survival (Bortolotti 2006).

The research topic initiated by Alfred Wallace has been a prolific and attractive area for many years. However, these days, one of the most rewarding subjects in evolutionary biology is Darwin's perspective on the function of colouration. Both mate choice (intersexual selection) and direct competition among individuals of the same sex (intrasexual selection) work as huge selective forces shaping a colourful array of signals of individual quality (Andersson 1994; Berglund et al. 1996).

These signals can be classified according to their origins, which often also determine their functions, given their different production costs (Badyaev & Hill 2000; Fitze & Richner 2002; Senar et al. 2003).

Some plumage colour traits with signalling properties are solely based on the micro-structure of feathers (Prum 2006), while others are based on different pigments (Hill & McGraw 2006). The most prevalent pigments in the avian integument are melanins, which colour mainly dark and brownish traits, and carotenoids, which usually result in yellow, orange or red colourations (Hill & McGraw 2006).

Melanin pigments are ubiquitous in nature, they are synthesized by all kinds of organisms (including bacteria, plants, fungi and animals) from the amino acids tyrosine and phenylalanine (Hearing 1993). In vertebrates, the synthesis of these pigments takes place in the epidermis, in structures known as melanocytes, and the process follows a precise coordination that leads, in birds, to the deposition of melanin pigments into keratin, through the feather follicle (McGraw 2006). There are two main forms of melanin, called eumelanin and phaeomelanin. The ratio of these two compounds in feathers determines the reflectance spectrum of the trait, such that feathers with a higher eumelanin content will be darker, while those with more phaeomelanin will be from brownish, to reddish, orange, or even yellow (Prota 1995; Toral et al. 2008). However, the final colour of the trait will be determined by the absolute amount of eumelanin, given its overwhelming effect on the reflectance spectrum (Jawor & Breitwisch 2003). Melanin production is partially under genetic control (Buckley 1987) although, the environment also seems to exert an influence on melanin colouration, specially through oxidative stress (Galván & Alonso-Alvarez 2009).

Carotenoids, on the other hand, are pigments only synthesized by plants, algae and fungi. Animals cannot produce them *de novo*, but have to ingest them in the diet (Goodwin 1984). The most common carotenoids found in bird colouration are lutein, zeaxantin, cryptoxantin and β-carotenoids (Stradi 1998). To colour their feathers, birds deposit carotenoids in the follicles, sometimes transforming the compounds before the deposition. This transformation, usually performed through oxidation, produces new pigments that add variation to the colouration (McGraw et al. 2003).

Given the different origins and paths followed by these pigments on their way to colour feathers, it has been hypothesized that different components of quality could be transmitted through different types of ornaments (Møller & Pomiankowski 1993). Selection may have linked each kind of ornament to the qualities that are best reflected by the expression of those pigments (Møller & Pomiankowski 1993). Hence, according to their different characteristics, while carotenoid ornaments should convey information about endoparasitism and immune ability, melanin ornaments are best suited to reflect information about nutritional health, ectoparasitism and hormonal levels related to agonistic behaviour and intrasexual competition. This differentiation has often been observed, and gives support to the existence of multiple colour traits that convey different qualities of their bearers through those different pigments (Candolin 2003). However, clarifying which factors determine the reliability of melanins and carotenoid as quality signals is currently growing in interest (Griffith et al. 2006; Hill & McGraw 2006; McGraw 2008). It is argued whether the condition dependence of melanin traits is significantly different from that of carotenoid based on carotenoids and, consequently, if these different traits could actually signal similar kinds of qualities (Griffith et al. 2006). Further research is therefore required on the possible different functions of melanins and carotenoids. According to some authors (Dale 2006; Griffith et al. 2006), the best way to tackle this problem would be to study species in which both kinds of ornaments are present, testing jointly the different functions of those ornaments in the same species and conditions.

The Eurasian siskin (*Carduelis spinus*; Box 1) and the great tit (*Parus major*; Box 1) are

two passerine birds that show ornaments based on melanins and carotenoids, which makes them a particularly suitable models for the study of multiple signalling. In this thesis, several experiments were performed on these bird species to achieve the objectives described in the next section.

BOX 1. Model species

Eurasian siskins (*Carduelis spinus*) are small, sexually dimorphic, finches that weight around 14g as adults. Females are grey-greenish, while males show more yellow plumage and a distinctive melanin based black bib. Siskins also present a carotenoid based yellow wing stripe. They are a social species, living in groups of 3-10 individuals all year round (Newton 1972). These groups show well defined structures, where dominant and subordinate individuals have very distinct roles and enjoy different benefits and costs (Senar 1985). Additionally, some groups are "transient" and usually do not use the same foraging patch for more than a day. Other groups are "resident" and can stay in the same place for several months. Resident siskins are dominant over transient ones and show better condition (Senar et al. 1990, 2001).
Study site Siskins studied in the experiments were captured in a suburban area of Barcelona and kept in captivity in enclosures within the Natural History Museum of Barcelona.
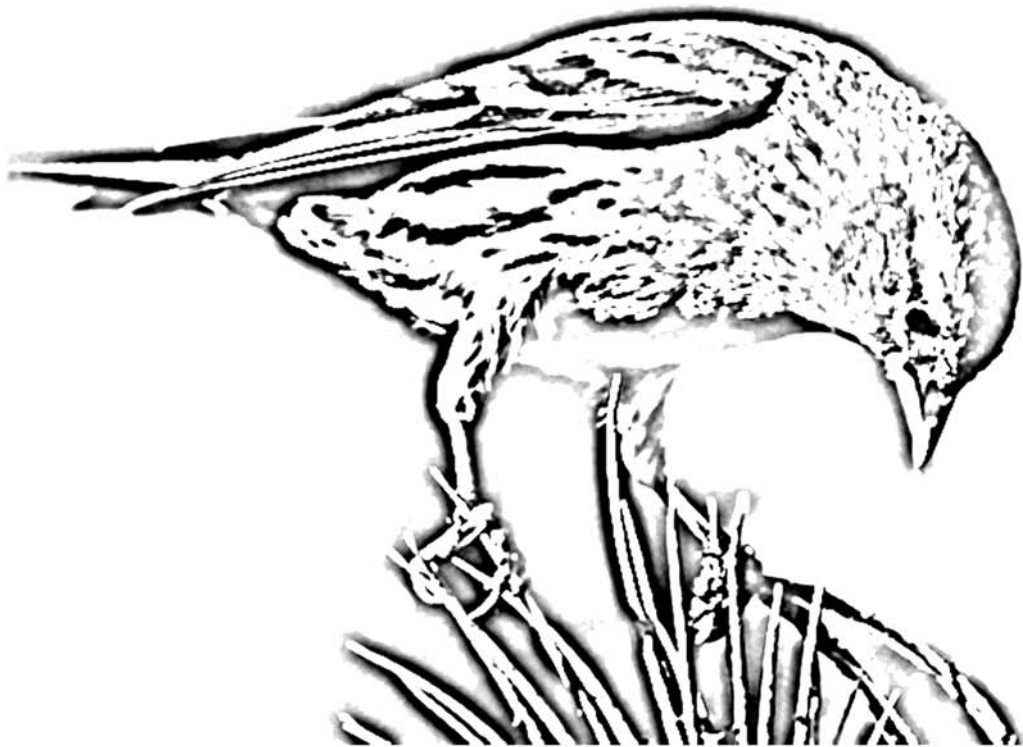
Great tits (*Parus major*) are non-migratory parids, weighting 17-21g as adults. They are socially monogamous birds that breed in holes and readily occupy nest boxes. European populations typically lay one or two clutches every breeding season (Gosler 1993). Great tits show high variability in their yellow carotenoid based ventral plumage. The yellow coloured breast is crossed by a melanin-based (Brush 1978) black stripe. This stripe reaches the legs in males, while disappearing gradually on the ventral side in females, allowing sex discrimination (Svensson 1975; Gosler 1993; Slagsvold 1993).
Study site The studied population breeds in Can Catà, a field station located in a Mediterranean mixed forest of deciduous oak (*Quercus cerrioides*) and aleppo pine (*Pinus halepensis*), near the city of Barcelona (northeast Iberian Peninsula; see Figuerola & Senar (2005) for more details). There are 180 nest boxes distributed in the study area (80ha), mainly occupied by great tits and some blue tits (*Cyanistes caeruleus*).

Objectives

- **Test the hypothesis that different colourations can signal different qualities of the individual**, allowing multiple criteria in sexual (and social) selection (Candolin 2003). Two traits that can greatly determine the response of individuals to environmental variation will be studied: Personality and Cognition.

- **Examine how multiple signals based on different colourations affect extra pair sexual behaviour.** This important component of sexual selection is considered a key mechanism to overcome the negative consequences of changing environments (Botero & Rubenstein 2012).

- **Explore how multiple characteristics of the individual interact to determine mate choice.** How much do animals trust colour signals versus their own experience? Given that fluctuating conditions could hamper the reliability of some quality signals, females may rely more on a direct continuous evaluation of prospective mates than in signals of quality (e.g. plumage colouration). A potential role of familiarity in mate choice could be especially relevant in changing environments.

Chapter 1: Carotenoids and foraging ability

Given the inability of animals to synthesize carotenoids, colour traits based on these pigments have long been considered a signal transmitting information about the foraging ability of the individual (Endler 1980). However, this general "ability to find food" has never been properly defined. In the siskin, the length of a carotenoid-based yellow wing stripe is used by females when selecting a mate (Senar et al. 2005). We predicted that this carotenoid trait would reflect the performance of an individual when solving a foraging problem, as a general measure of foraging ability.

To test this prediction, we examined how differently ornamented male siskins performed in accessing food from a partially blocked feeder. The task required birds to remove one or two toothpicks from the feeder in order to reach some pine seeds below. We used the time taken to access the food as a measure of performance.

As expected, male siskins with longer yellow wing stripes were faster at obtaining food from a small box whose access was blocked by toothpicks (Paper 1, Figs 1 & 2).
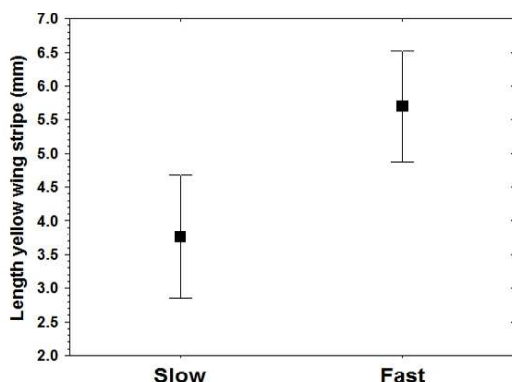
Figure 1: Differences in the length of the yellow wing stripe, measured on primary six (n=29), between slow and fast problem solvers. We defined slow solvers as birds that either did not solve the problem or needed more than one trial to solve the problem (greater than 300 s). Fast solvers are defined as birds that solved the problem within the first trial (less than 300 s). Fast solvers displayed longer yellow wing stripes than slow solvers (s.e. bars shown).

We thus showed that the length of the carotenoid-based yellow wing stripe of siskins could be a signal of the ability to solve a foraging problem. This trait, however, did not reflect the latency to approach the problem, which discards any neophilia effect. The approach we used to test their problem solving ability may be considered not to measure all aspects of this ability and to be rather artificial. However, we chose this approach because evolution cannot have provided animals with a built-in solution for novel situations of this type, and these animals have not had a chance to learn what to do through trial-and-error. This explains why older individuals were
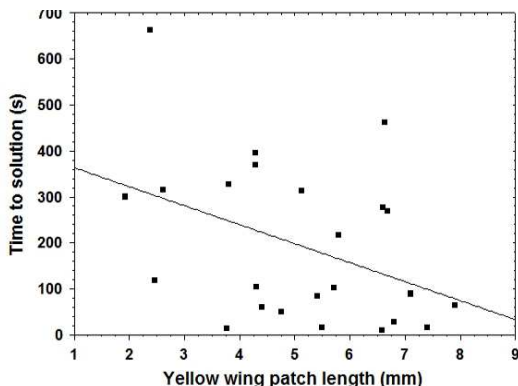
Figure 2: Relationship between the length of the yellow wing stripe (n=21) and the time taken to solve the foraging problem. Birds with longer yellow wing stripes solved the problem faster (table 1). The figure only includes birds that solved the problem.

Table 1: Cox proportional hazards analysis relating length of the yellow wing stripe, size of the black bib, age and size of the bird (tarsus length) with the time taken to solve a foraging problem (n=21). (Interactions were not included since they were not significant.)

Variable	Coef	Exp(Coef)	S.E.(Coef)	Z	p-value
Yellow wing stripe	0.38	1.47	0.18	2.13	0.03
Black bib	20.94	0.39	0.60	21.57	0.12
Tarsus	20.37	0.69	0.56	20.66	0.51
Age	20.02	0.98	0.02	21.10	0.27

not able to take advantage of their experience (Paper 1, Table 1). Hence, although we recognise that our results should be reinforced by testing additional foraging problems, the performance showed while solving this novel task is likely to reflect an overall ability of the individual to solve problems (Chappell & Kacelnik 2002). Challenges of this type are not uncommon in nature, and birds are known to develop singular feeding innovations (Lefebvre 2000). A classical example is the ability of wild tits to manipulate and open milk bottles (Fisher & Hinde 1949).

Female choice for cognitive traits

Traditionally, the idea that carotenoid-based colouration is related to foraging ability had been tested from the framework linking diet to colouration (Hill 2006). Here we show that acquiring carotenoids to colour ornaments is not only a question of availability and ingestion but also of ability to obtain them. Moreover, while we do not know yet the breadth of the task domain over which our index applies, it also appears that, at least for one dimension of problem solving, females' choice criteria may be adapted to gauge cognitive performance.

The study of mate choice for cognitive traits has been addressed only very recently (Keagy et al. 2009; Boogert et al. 2011) and it offers a promising research topic. The study of sexual (and social) preferences for cognitive traits is particularly relevant given the potential role of cognition in the adaptation to novel or changing environments (Reader & Laland 2002; Sol et al. 2002, 2005, 2007; Ratcliffe et al. 2006).
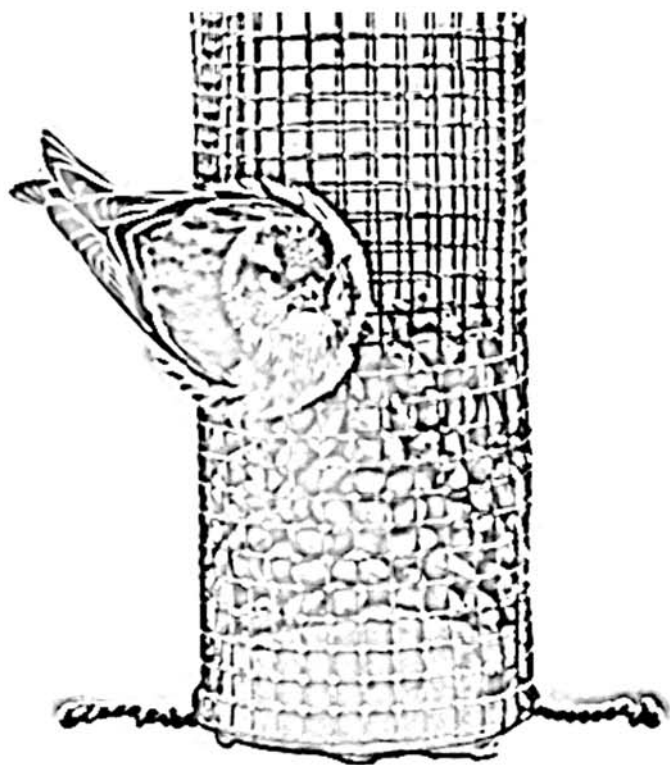
Theodore Roosevelt, Life-Histories of African Game Animals

"The darker the night, the bolder the lion."

Chapter 2: Melanin and personality

What is personality?

The way by which individuals respond to novel situations, evaluate potential risks or drive social interactions can have an obvious effect in many aspects of their lives. A certain behavioural flexibility would confer adaptation advantages in changing environments but, very often, what we actually see in nature is a lack of plasticity in behaviour: Animals respond consistently on the same way in different situations, which could even be labelled as maladaptive (Sih et al. 2004). These behavioural differences, that are consistent over time and across situations are what we know as personality (Réale et al. 2007).

Even though some authors addressed animal personality as early as in the seventies (Huntingford 1976), this topic has been largely ignored until recent years (Réale et al. 2007). Currently, we are witnessing a huge rising interest in personality among behavioural ecologists, but our understanding of the subject is still scarce (Réale et al. 2010). However, there is now enough evidence to affirm that personality traits have clear fitness consequences (Dingemanse & Réale 2005; Smith & Blumstein 2007). Personality can affect a variety of capital ecological variables, such as dominance, natal dispersal, offspring recruitment, territory quality, reproductive performance, survival and physiological responses to social stress (Réale et al. 2007). All these traits may have a strong effect on fitness (Dingemanse & Réale 2005; Smith & Blumstein 2007). For example, a bolder individual might be more prone to reach a dominant position, gaining access to resources. However, this boldness could jeopardize his survival if it makes him more conspicuous to predators.

Personality traits do not always act independently. Often, behavioural traits (Andersson 1994) are correlated. For example, bolder great tits (*Parus major*) are also more aggressive and faster explorers (Verbeek et al. 1994; Drent et al. 2003). These suites of correlated behaviours are known as behavioural syndromes, and can dramatically increase the effect of personality on fitness, due to higher constraints on behavioural plasticity (Sih et al. 2004).

Given the advantages (and disadvantages) that certain personality traits might confer in different situations, it has been proposed that personality can be maintained and selected by means of natural selection (Dingemanse & Réale 2005). But also, an individual's success may be affected by the personality of its mate. Consequently, sexual selection has been proposed as a way to maintain personality variability in nature (Schuett et al. 2010).

Could personality be signalled?

Individual qualities that increase fitness are often signalled by sexually selected traits, such as bright colours, enlarged fins, feather plumes, song or horns (Andersson 1994). If personality traits are also sexually selected individual qualities, it would be expected that signals conveying information about personality traits have evolved adaptively. In a social selection context, these signals could also play an important role, for example, when selecting or avoiding a social companion (Tanaka 1996; Senar & Camerino 1998).

Animals base their decisions either on their own experience or following other individuals' example, i.e. using social information (Danchin et al. 2004). Given that personality can affect, for example, different aspects of foraging performance (Schuett & Dall 2009; Exnerová et al. 2010; van Overveld & Matthysen 2010), individuals using social information would benefit greatly from being able to identify personality signals and use them to evaluate social information.

In spite of the potential biological importance of personality signals, the study of this topic has only very recently been addressed. At least two possible channels have been identified with potential to transmit information about personality traits: Bird song (Garamszegi et al. 2008)

and melanin colouration (Ducrest et al. 2008). Given that melanin based colourations are ubiquitous in nature, the study of personality signals based on this trait is highly relevant.

Melanin colouration and personality

Animal personality is, at least to some extent, gene regulated and heritable, as suggested by the results of artificial selection experiments (Drent et al. 2003). Still, some physiological processes could have an additional influence on behavioural traits. One potential process is the regulation of the melanin synthesis. Melanin is the main pigment in charge of the colouration of brown-black traits (McGraw 2006). The synthesis of this pigment is argued to be partially influenced by the environment, both by the environmental availability of melanin precursors and by differential effects of oxidative stress (Galván & Alonso-Alvarez 2009). However, the system is also controlled by genes that regulate the hormonal pathways of the melanin synthesis (Buckley 1987). Ducrest et al. (2008) proposed that the melanocortin-system, the hormonal system in charge of the synthesis of melanin, could affect behaviour, via pleiotropic effects. Melanocortins are hormones that bind to five different receptors. These receptors link to very different functions and could affect several phenotypical and behavioural traits (Ducrest et al. 2008). This hypothesis was supported by earlier studies that found that darker individuals often are more aggressive, more sexually active and more resistant to stress than lighter individuals (Roulin et al. 2000; West & Packer 2002). Recently, this support has been strengthened by a few studies showing different links between melanin and personality in a number of species: salmonid fishes (Kittilsen et al. 2009), eastern Hermann's tortoises, *Eurotestudo boettgerii* (Mafli et al. 2011) and barn owls *Tyto alba* (van den Brink et al. 2011).

Additional comparative work in other species would help to reveal whether the often observed link between aggressiveness and melanin colouration has been evolutively conserved, or appeared through convergence processes (Price et al. 2008). Also, the increasing evidence that personality signals based on melanin traits are widespread in animal species opens a highly relevant research topic. In conclusion, a test on the possible signalling of personality through feather colouration, in a species showing both melanin and carotenoid-based traits, would help to reveal the different role of those pigments. In this thesis, we performed such a test in the siskin (*Carduelis spinus*; Paper 1).

Signalling of personality in the siskin through melanin traits

Siskins show dark feather traits based on melanins. The black bib of the siskin works as a social signal, whose size reflects the dominance of the individual within the social group (Senar et al. 1993; Senar & Camerino 1998). The size of this bib is also correlated with aggressiveness (Senar 1985), a behavioural trait (Box 2) often related to boldness (Sih et al. 2004) and exploratory behaviour (Dingemanse & de Goede 2004; Bell & Sih 2007), but see (Jones & Godin 2010). Therefore, we would expect that siskins with larger bibs would also show higher levels of exploration. We tested this hypothesis by measuring the exploration scores of a group of siskins, following Verbeek et al. (1994), and relating those exploration scores to their black bib sizes (Paper 2).

As predicted, male siskins with larger black bibs showed a shorter latency time to approach a novel object (Paper 2, Fig. 3), demonstrating a higher exploratory behaviour.
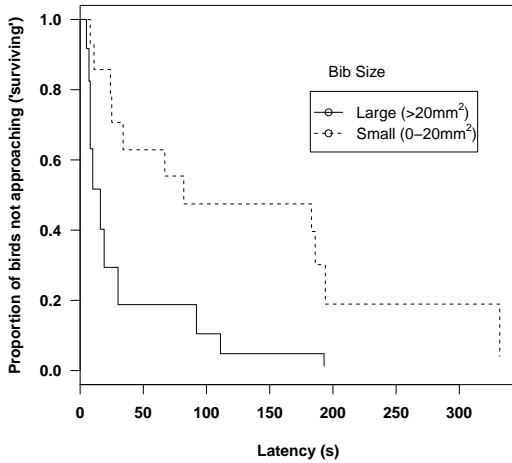
Figure 3: Survivorship plot function for tendency to approach a novel object. It shows the proportion of siskins that did not approach the object ("surviving") up to the respective time interval. Birds (n=28) are represented according to the size of their black bib, which shows a high bimodality in the population (Ripoll et al. 2004).

BOX 2. Personality categories

Behavioural ecologists have agreed to classify behavioural differences into 5 different major temperament traits categories: exploration-avoidance, shyness-boldness, activity, sociability and aggressiveness.

The first axis, **exploration-avoidance**, describes the reaction of the individual to novel environments or situations. Some individuals explore new environments fast and superficially, while others do it slower but more thoroughly. Part of this personality axis are traits like neophilia or neophobia. The second axis, the **shyness-boldness** continuum, describes the individual's reaction to a potentially risky situation that is not new, like a predator encounter, for example. The third one is the measure of **activity** of the individual. This trait can interfere with the two previous ones and, when studied, it should be distinguished between novel or risky situations. **Aggressiveness** reflects the agonistic behaviour of the individual towards conspecifics and, finally, **sociability** describes the individual's predisposition to interact with conspecifics, excluding agonistic behaviour (Réale et al. 2007).

Why would siskins with a larger black bib approach a novel object faster?

Melanin-based colourations are linked to several physiological, morphological and behavioural traits (Roulin 2004). It has been proposed that these links originate in the pleiotropic effects of the genes regulating the synthesis of melanin (Ducrest et al. 2008). Accordingly, it has been found that, for example, darker eumelanic barn owl nestlings cope better with stress, releasing less corticosterone after a stressful event (Almasi et al. 2010). Dominant siskins, bearing larger eumelanic bibs, also seem to handle stress better than subordinates, according to their metabolic rate (Senar et al. 2000). This increased ability to cope with stress could be the reason behind their faster response to a novel object and should thus reflect a superior ability to handle, for example, the stressful factors of a different environment (Almasi et al. 2008; Roulin et al. 2008).

Selection for personality signals

Siskins with larger black bibs have been found to be dominant within the group (Senar et al. 1993; Senar & Camerino 1998) and also more aggressive (Senar 1985). Foraging siskins avoid conspecifics showing larger black bibs, probably due to prevention of potential aggressive interactions (Senar & Camerino 1998).

But most likely, siskins are not only trying to keep away from aggressive larger black bib owners. Signalling of different personalities could help to shape foraging tactics, such as the producer–scrounger game (Barnard & Sibly 1981), which has been found to be affected by personality (Kurvers et al. 2010a). Slow explorers, signalled by small bibs, apart from being less aggressive, are lesser risk-takers (van Oers et al. 2004) and their personality helps them to quickly identify and avoid potentially dangerous aposematic prey (Exnerová et al. 2010). Despite their higher wariness, slow birds are active foragers that explore their environment more thoroughly than faster birds (Verbeek et al. 1994). They could therefore be a potential source of high quality social information and this additional value could be assessed a priori using a personality signal. Hence, probably siskins are not only trying to keep away from aggressive individuals with large bibs, but rather actively selecting smaller black-badged, slow-exploring individuals as social companions.

Could there then be some selective advantage for dominant, fast-explorer birds?

These dominant, fast explorers with large bibs could have a potential survival advantage, owing to their superior access to resources. However, in the siskin, dominant individuals mainly fight among themselves, in like-versus-like aggression (Senar et al. 1989, 1997; Ripoll et al. 2004) leaving lower-ranking individuals relatively free from aggression. Also, female siskins do not choose mates based on this trait (Senar et al. 2005) and, accordingly, theoretical models suggest that the relationships among survival, reproduction and aggression rates of dominants and subordinates probably maintain the evolutionary stability of the system. This is nicely reflected by the binomial frequency distribution of bib sizes in nature (Ripoll et al. 2004). Additionally, empirical studies suggest that, in these "feudal" species, in which dominants fight only among themselves (Senar et al. 1990, 1997), both behavioural classes achieve similar survival rates (Ripoll et al. 2004; Senar & Domènech 2011), so it is likely that these are **two alternative, equally valid strategies**.

Finally, **plasticity in selection pressures** on different personality traits, originated from changes in, for example, environmental conditions (Dingemanse et al. 2004), could help to maintain the genetic variation in personalities.

The importance of playing second fiddle:
Multiple signals and extra pair sexual behaviour

Chapter 3: The importance of playing second fiddle: Multiple signals and extra pair sexual behaviour

In the two previous chapters we have confirmed that different colourations can signal different qualities of the individual. Multiple signalling allows for flexible selection, a process that could increase the odds of survival and fitness success in changing environments (Bro-Jørgensen 2010). Also, the potential existence of several successful combinations of qualities sets the grounds for the flourishing of different strategies, a topic that could be especially interesting in sexual selection contexts (Moran 1992).

Extra pair sexual behaviour is argued to be one of the major sources of sexual selection on male sexual secondary characters, particularly in monogamous mating systems. In birds, extra pair paternity variation is considered an important process behind the evolution of sexual dichromatism (Møller & Birkhead 1994; Sheldon & Ellegren 1999).

Extra pair sexual behaviour provides several sex-dependent advantages. For males, it means a direct source of fitness, with no parental involvement. For females, it is a way of gaining genetic variability and quality, while keeping parental help from the social partner. For both sexes, it is an assurance against the potential infertility problems of their first mate choice (Birkhead & Møller 1992). In short, extra pair sexual behaviour allows a higher flexibility in sexual selection, given that it offers a second chance to make a better choice. Secondary sexual choices could have a role in preventing, correcting or reducing the negative consequences of ecological crossovers, that is, the possibility that fluctuating environments change the relative quality or sexual attractiveness of potential mates (Botero & Rubenstein 2012).

In this chapter, we examined the role of multiple colour signalling in extra pair behaviour, by using a socially monogamous dichromatic species in which there is strong evidence for the use of multiple signals: the great tit (Senar et al. 2003, 2008; Ferns & Hinsley 2008; Senar & Quesada 2006; Rivera-Gutierrez et al. 2010). Particularly, we studied the effect of melanin and carotenoid multiple signals in wild male great tits on (1) the agonistic behaviour against conspecific territorial intrusions and (2) the risk of suffering cuckoldry.

Given that male great tits with a larger black tie size are known to defend more strongly their nests against predators (Quesada & Senar 2007), we predicted that the same trait would determine the strength of the response also against conspecific intrusions. Consequently, those males showing larger black ties should have lower risks of suffering cuckoldry.

According to our prediction, males with a larger black tie area defended their nests more intensely against a conspecific intrusion. However, we found an interaction between the chroma of the yellow plumage of the chest and the size of the black tie. This interaction was such that, while the expression of each trait by itself reflected a higher defensive response, higher levels of the two traits in combination resulted in a reduced aggressive behaviour against conspecific intruders. Individuals whose melanin and carotenoid traits signalled the highest quality defended their nest significantly less than individuals showing high expression levels of only one kind of trait (Table 2, Fig. 4).

Why did not carotenoid colouration have an effect in previous studies where the response against predator intrusions has been investigated?

Quesada & Senar (2007) did not find any such effect of the yellow breast colouration on the response against a predator. This fact could be due to the different nature of the intrusions investigated. In the face of a predator attacking its nest, a great tit has only two simple alternatives: to risk his life trying to save his offspring or to let them die. In this situation, probably only his own aggressiveness (reflected on his melanin-based trait (Järvi & Bakken 1984;

Table 2: Final model of multiple backward regression of territorial nest defence (PC1) on black tie size (mm^2), chroma of the carotenoid-based yellow plumage of the chest and interaction between the two. $F_{3,19}=7.49$, p=0.002.

Variable	Estimate	SE	t	p
Black tie size	-0.09	0.02	-4.05	<0.001
Chroma	-1.23	0.36	-3.43	0.002
Chroma*Black tie size	0.004	0.001	3.77	0.001
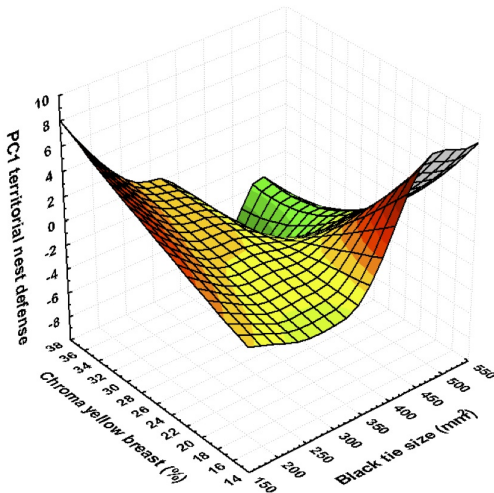
Figure 4: Relationship between nest defence (PC1), chroma of the yellow chest and black tie size (mm^2).

Quesada & Senar 2007), and its potential loss of investment, in terms of brood size, will have an influence on his decision (Quesada & Senar 2007). Accordingly, individual qualities such as foraging abilities, immunocompetence or parasitic resistance, which are signalled by yellow carotenoid-based colouration (McGraw & Hill 2000a, b; Senar et al. 2003; McGraw & Ardia 2007), may be irrelevant in this extreme situation. On the side of the predator, this conspicuous yellow colouration will not very likely make a difference either, given the dissimilarity in colour vision between these species and common predators (Håstad et al. 2005). This may explain the lack of association between carotenoid-based colouration and nest defence in front of predators found previously (Quesada & Senar 2007), is expected. However, a conspecific intrusion may not be such a drastic event, assuming a lower risk for the resident male and his offspring. In this situation, his investment in terms of brood size would not be so critical. Accordingly, in opposition to Quesada & Senar (2007), our experiment showed that the number of nestlings did not have an effect on the male's response.

Why do individuals of higher quality, reflected both on carotenoid and melanin traits, not defend their nests against conspecific intrusions?

A possible explanation could be that, as in blue tits (*Cyanistes caeruleus*), females paired to males of high quality keep faithful to them (Kempenaers et al. 1992, 1997), so they would not need to invest energetic resources in nest and mate guarding. To test this hypothesis, we also performed paternity tests.

However, when relating male colouration to the risks of suffering within nest paternity loss,

our results did not support the initial hypothesis. We found that male great tits with larger black ties and higher levels of chroma on their yellow plumage suffered higher rates of cuckoldry than those showing high expression levels of only one of those colour traits. Birds showing lower expression levels in both traits also suffered comparatively more cuckoldry (Fig. 5, Table 3).
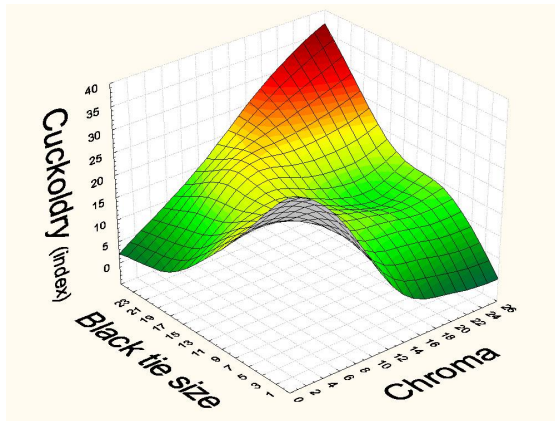

Figure 5: Proportion of extra-pair/within pair paternity in relation to size of the black tie and chroma of the yellow breast colouration of male great tits.

Table 3: Ranked GLM relating size of the black tie, yellow chroma, and their interaction with the proportion of extra-pair/within pair paternity of male great tits (Final model).

	F	β	p
Black tie size	5.88	-1.10	0.02
Yellow chroma	5.66	-1.22	0.03
Black tie size*Yellow chroma	8.32	1.21	0.01

Surprisingly, to show high quality colour traits did not only not prevent their bearers from being cuckolded, but led them to suffer even higher cuckoldry rates than less ornamented individuals.

A possible interpretation for these results could be that, in our population, males might be following different strategies according to their individual quality. In great tits, males perform costly paternity assurance behaviours, like mate guarding (Björklund & Westman 1986; Björklund et al. 1992) and song at dawn (Mace 1987). In many species, males showing higher quality traits usually enjoy bigger chances of getting extra pair copulations (Birkhead & Møller 1992; Bitton et al. 2007; Balenger et al. 2009), even regardless of the quality of the male they are contributing to cuckold (Forstmeier 2007). With this promising prospect, attractive male great tits could find more profitable to invest more in pursuing extra-pair copulations, instead of employing their efforts in those costly mate guarding behaviours, even if that increases the chances of compromising their within pair paternity (Kokko & Morrell 2005). In fact, in great tits, a perceived loss in within pair paternity leads in turn to an even lower mate guarding effort (Hansen et al. 2009).

This same strategy has been suggested for some species in which older males suffer more cuckoldry than younger ones (Westneat 1987; Sherman & Morton 1988). It is also a tactic known to be adopted, for example, by black-throated blue warbles *Dendroica caerulescens* (Chuang-Dobbs et al. 2001) where males with more extra-pair opportunities guard less and suffer more cuckoldry.

The alternative strategy would be the one followed by males showing only high levels of either their melanin or their carotenoid based trait. These individuals of a suboptimal attractiveness,

either based on a high yellow chroma or a large black tie size, seem to suffer less cuckoldry in our population. Probably for these males it would be more beneficial to ensure their within-pair paternity by enhancing their mate guarding strategies, given that they would not be so successful when trying to achieve extra-pair copulations. In fact, it has been suggested that less sexually attractive individuals seem to follow an alternative strategy by increasing their aggressiveness (McGraw et al. 2007), mate guarding (Johnsen & Lifjeld 1995), and parental care (Sanz 2001). This higher parental effort could be rewarded in faithfulness by females (O'Brien & Dawson 2010), which would again, in turn, reinforce the male's investment in the nest (Lubjuhn et al. 1993; Lubjuhn 1995).

Lastly, those males with small tie sizes and low chroma values, signalling a very low quality, could not expect to successfully achieve many extra-pair copulations. Females paired with these low quality males may be actively seeking for EPCs (Kempenaers et al. 1992; Kempenaers 1995) but, even if these males decide to follow a mate guarding strategy, their low condition (i.e. low yellow chroma (Senar et al. 2008) and low aggressiveness (Senar 2006; Galván & Alonso-Alvarez 2008) would result in a poor nest defence/mate guarding performance. This would probably lead to the observed paternity loss of these males in our population (Kempenaers 1995).

In conclusion, we found that males of the highest quality, as signalled by carotenoid and melanin based colour traits, defend their nests less against conspecific intrusions (Paper 3) and, possibly in consequence, are as heavily cuckolded as those with lowest quality traits (Paper 4). Males of intermediate quality seem to be more successful at keeping their within pair paternity, probably because of their stronger nest defence against conspecifics.

These results suggest that males might be following different reproductive strategies according to their individual quality. Unfortunately, our data did not allow us to identify the genetic father of the extra pair offspring. This information would confirm whether male great tits with both melanin and carotenoid-based high quality traits are indeed successfully achieving more extra-pair copulations, a prediction that arises from our results and should be tested in future works.

Aesop, The Jay and the Peacock.

"It is not only fine feathers that make fine birds"

Chapter 4: Beyond signals: Better the devil you know?

In Chapter 3 we observed that multiple signals potentially could allow different strategies in extra pair sexual behaviour. Both multiple signalling and extra pair sexual behaviour have been recently proposed as mechanisms to overcome the negative consequences of changing environments (Bro-Jørgensen 2010; Botero & Rubenstein 2012), for example, by allowing a more dynamic social selection and a "choice adjustment" when circumstances change.

However, even with the aid of the information conveyed in multiple signals, a secondary choice (e.g. extra pair sexual behaviour, divorce and subsequent mate choice for a second clutch, or just refining a social companion choice) could fail when, for example, unexpected environmental changes occur. If the conditions at the time of sexual or social selection are different than those at the time of signal producing, the value of the signals as indicators of quality could be severely damaged. The same result would be obtained if the quality signals of different individuals have been produced in separate habitats. For example, in species that are allopatric during the breeding season but meet again in winter to pair (Borras et al. 2010, 2011).

Consequently, despite the informative potential and the obvious importance of quality traits, such as colour signals, animals should be expected to allocate much of their trust in direct proofs of value, when making decisions on social or sexual matters. That is, animals should trust their own experience, even over the information obtained from other individuals' quality signals. In case of doubt, they should prefer what they already know.

In this last chapter (Paper 5) we examined the possible limitations of colour signals in sexual selection, performing two mate choice experiments in siskins, a species in which females use the length of the yellow wing stripe to select their mates (Senar et al. 2005).

In the first experiment, we tested the preference of female siskins when choosing between familiar and unfamiliar males, with similar sized yellow wing stripes. Our prediction was that, from two males with similar sexual signals, a female siskin would prefer the familiar one.

In accordance with our prediction, females clearly selected the known male over the unfamiliar one (Fig. 6, top).

But, how strong is this preference for a familiar male? In a second trial, we determined the relative strength of familiarity and ornamentation, by confronting females with the choice between a highly ornamented unfamiliar male and a less ornamented familiar male. In this experiment, female siskins did not show any significant preference (Fig. 6, bottom).

Previous studies analysing the interaction between male attractiveness and social familiarity in mate choice found that familiarity bred contempt and that attractiveness was more important than familiarity when choosing a mate (Patricelli et al. 2003; Zajitschek et al. 2006; Zajitschek & Brooks 2008). The results from our study in siskins support a new view, in that attractiveness alone is not enough in some species and that previous experience is an added value that could interact with quality signals.

The familiarity factor may help us understand patterns of sympatric local adaptation and divergent ecological selection. This factor may be especially important in species in which different subpopulations cohabit during the mating period, since this cohabitation would otherwise provide ample opportunities for gene flow (Schluter et al. 2001; Newton 2008). Crossbills *Loxia* spp. are considered a typical example; studies have shown that different cryptic species may cohabit the same geographical areas but seem to mate assortatively (Benkman 1993; Groth 1993; Parchman et al. 2006; Snowberg & Benkman 2007, 2009; Summers et al. 2007; Edelaar et al. 2008). The citril finch *Serinus citrinella* is another example. Different subpopulations, known to adapt locally and to differ in morphometrics and genetic structure (Senar et al. 2006; Forschler et al. 2011), are allopatric during the breeding season but intermix in the shared wintering areas where pairing takes place (Borras et al. 2010, 2011). Citril finch subpopulations, however, remain differentiated (Senar et al. 2006; Forschler et al. 2011). The siskin is a third
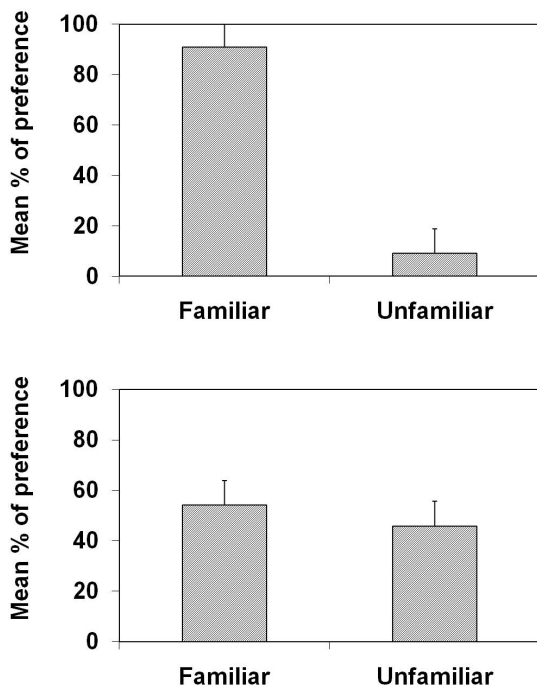
Figure 6: Results from the two experiments in which the siskin females were presented with a choice between two male siskins differing in social familiarity (Familiar and Unfamiliar) and sexual attractiveness, measured as the relative length of the yellow wing stripe. In the first experiment, females had to choose between a familiar and an unfamiliar male, both ornamented with a large yellow wing stripe (both attractive). In the second experiment, females had to choose between a poorly ornamented familiar male (small yellow wing patch) and a more ornamented unfamiliar male (large yellow wing patch). Results are expressed as the mean percentage (±S.E.) of time that the test female stayed with either stimulus male during a total of 4 hours (14.400 s.). (n=15 trials for both experiments).

example. Two different subpopulations, residents and transients, have been described in this species (Senar et al. 1992). These two subpopulations intermix and cohabit during the winter pairing period (Senar et al. 1990, 1992), but subpopulations consistently differ in morphometrics (Senar et al. 1994, 2001).

Subpopulations remain differentiated in crossbills, citril finches, and siskins, even though cohabitation of the different subpopulations during the pairing period would provide ample opportunities for gene-flow (Choudhury & Black 1994; Borras et al. 2011). Here, we stress that a mating preference for socially familiar individuals, as found in the present study, could nicely explain the maintenance of these instances of local differentiation (Choudhury & Black 1994; Borras et al. 2011).

Interspecific variation in the role of familiarity in mate choice has mainly been attributed to the probability of inbreeding (Cheetham et al. 2008). This would explain why, in species with a high probability of inbreeding, as for instance promiscuous species, females generally avoid males that are familiar to them (Cheetham et al. 2008). However, we think that interspecific variation in the role of familiarity in mate choice could also be related to interspecific variation in the degree of stability in the composition of groups, which is in fact a prerequisite for social familiarity. Cardueline finches, like crossbills, citril finches and siskins, breed in semi-colonies that have high social interaction among their members (Newton 1972; Glück 1980). The high social costs of flock switching enhance membership stability (Balph 1979; Mundinger 1979; Senar et al. 1990; Cristol 1995; Wilson 2006), and data suggest that flocks formed in the original breeding localities may even remain together in the wintering grounds (Wilson 2006). In this scenario, if birds develop a mating preference for familiar birds, flock structure would favour pairing with individuals from the same breeding subpopulation, thereby reducing gene-flow and allowing local adaptation.

There is increasing evidence that animals may use multiple criteria in mate choice (Candolin

28

2003). Our present results suggest that in some species, quality signals are not always blindly followed, and social familiarity adds to attractiveness. As preference for familiar individuals as mates could favour local adaptation, social familiarity should be regarded as an important selection pressure in modulating the evolution of characters.

Carlos Cuerpo Caballero

"Lo importante es que podemos elegir."

Do melanin and carotenoid based traits signal different things?

Griffith et al. (2006) suggested, based on results from a meta-analysis, that there was not enough evidence to determine that melanins and carotenoids have different signalling functions due to their different origins. It was proposed then, that more experimental data on species bearing both kinds of pigmentations could help to prove this hypothesis.

Our results (Papers 1 & 2) support this view: While the carotenoid based yellow wing stripe independently reflected the foraging ability of male siskins, the melanin based black bib was the only signal related to a personality trait in the same species, being both traits uncorrelated (Senar & Escobar 2002, unpublished data)

But, is this really universal? Could there be any role of, for example, carotenoid colouration in personality signalling? Actually, we could not totally discard a potential, context-dependent, correlation between personality traits and carotenoid colouration. For example, due to the effects that personality can exert on foraging behaviour (Verbeek et al. 1994; Kurvers et al. 2010a; van Overveld & Matthysen 2010; David et al. 2011; Cole & Quinn 2012), some personality traits could provide foraging advantages in some contexts or populations. Given that foraging success is often reflected in an enhanced carotenoid-based colouration, competitive personality traits could eventually correlate to this kind of colourations.

A potential example could be the guppy (*Poecilia reticulata*). Males of this small fish show bright orange spots on their body (Houde 1997), that female guppies use in mate choice (Kodric-Brown 1989; Grether 2000). Given that carotenoid intake affect the saturation of orange spots, it has been proposed that these orange traits are signals of the foraging ability of the guppies (Kodric-Brown 1989; Grether et al. 1999). Karino et al. (2007) performed an experiment in which male guppies had to explore a simple maze in order to reach the carotenoid-rich algae they usually feed on. Male guppies with more saturated orange spots were able to find faster the algae. These results were interpreted as a higher algal searching ability for those individuals showing more saturated orange spots. However, the experimental set up prevented them from separating foraging ability from exploratory behaviour. A higher exploration score could be responsible of the superior success of bright coloured guppies in this experiment.

There could be additional mechanisms maintaining the correlation between carotenoids and personality in the guppy. Female guppies are not only attracted to carotenoid-based signals. They show an even stronger preference for bold males, regardless of their colouration (Godin & Dugatkin 1996). As a result, there seems to be a positive relationship between the conspicuousness of the males and their boldness in the face of predators (Godin & Dugatkin 1996). This correlation could lead to the assumption that carotenoid based traits could work as signals of personality in the guppy. But in fact, most probably, this link is actually driven by the female preference for two different traits: personality and foraging ability.

Which one of those traits is honestly signalled by carotenoid colouration? To properly test the honesty of a signal, it is necessary to isolate each function to avoid confounding factors; in this case, to separate personality from foraging ability. Our experimental design allowed us to tackle this problem in the siskin and confirm that carotenoids (Paper 1) and melanins (Paper 2) do have different signalling functions in the siskin.

Colour signals, interactions and dynamic selection

As previously discussed, certain personality traits could also provide foraging advantages, although they are different from those granted by a higher problem-solving ability. Hence, while

a better cognitive performance can aid to solve foraging problems, personality can affect the chances to find those foraging challenges and the way to approach them. Furthermore, recent works also suggest that personality and cognition might have an even closer relationship. For example, in great tits, some personality traits can improve learning performance or competitive ability (Cole & Quinn 2012; Titulaer et al. 2012).

Given the different advantages of each trait, and their interactions, it is expected that signals informing about those different individual qualities have a shared influence when choosing a mate or a social companion.

For instance, while female siskins seem to select their mates mainly based on the size of the yellow wing stripe (Senar et al. 2005), foraging individuals tend to avoid conspecifics showing larger black bibs (Senar & Camerino 1998) and even, possibly, they actively choose those showing small black bibs. The length of the yellow wing stripe gives valuable information about the foraging ability of the individual, but siskins can also assess their companions' personality through their melanin traits, and adjust their choice. The availability of clues about different qualities could allow individuals to refine social and sexual selection, by following alternative strategies that suit different contexts and situations.

The best strategy: A matter of character?

One of the means by which animals can potentially correct or compensate suboptimal choices, in a sexual selection context, is extra pair sexual behaviour. In Chapter 3, our results showed that different combinations of traits where related to the intensity of nest defence (Paper 3), and implied differences in paternity loss (Paper 4). These findings suggest that males might be using different strategies to maximize their qualities.

Could personality drive these and other strategies? Recent studies show that extra pair paternity is related to exploratory behaviour in the great tit (van Oers et al. 2008; Patrick et al. 2011). Interestingly, in one of the populations studied, male fast explorers tended to gain more paternity outside their social pair, but that implied a loss of paternity within the social nest (Patrick et al. 2011). Exploratory behaviour is often related to activity levels, risk-taking and boldness (Groothuis & Carere 2005). These behavioural traits could help to explain the preference of different individuals for a certain reproductive strategy. However, given that extra pair sexual behaviour implies more than a single individual, future studies taking every part of the conflict into account are needed to disentangle this problem.

In Chapter 4, we observed that, very often, female siskins seem to trust their own experience over the information obtained from colour signals. Personality could also potentially interact with the use of signals at individual level. For example, personality is known to affect the use of social information: individuals showing certain personality traits might be more prone to follow other individual's example (Kurvers et al. 2010b). We could expect, then, that individuals bearing different personalities also show a different tendency to use the information obtained from quality signals, probably not only in social contexts, but also in sexual selection.

The study of the effect of personality in the function and use of colour signals will undoubtedly prove a prolific and interesting line of research.

Conclusions

- The carotenoid based yellow wing stripe of the siskin reflects the individual's ability to solve foraging problems. Evolution may have provided female siskins with tools to evaluate the cognitive performance of the males.

- The melanin based black bib of male siskins predicts their exploratory behaviour and could be used as a source of valuable social information.

- Colour signals based on different pigments have separate functions in the siskin, allowing multiple criteria in sexual (and social) selection (Candolin 2003).

- The different qualities signalled by melanin and carotenoid-based colour signals of male great tits seem to be implicated in intraspecific nest defence and extra pair paternity. The interactions observed between these signals suggest that male great tits might be following different reproductive strategies according to their individual quality.

- Familiarity adds to attractiveness when female siskins choose a mate. Our results stress that, at least in some species, females use multiple criteria in mate choice, not necessarily related to external signals of quality, such as colouration.

- Multiple qualities, signalled by different colour traits, have the potential to be part of different successful social and sexual mate choice strategies, aiding a more plastic selection. Our results support the hypothesis that selection plasticity could be one of the mechanisms behind the origin and maintenance of multiple signals (Bro-Jørgensen 2010).

Resumen en Castellano

Introducción

En procesos de selección sexual o social, los animales emplean diferentes estructuras para señalizar sus cualidades. El uso de señales múltiples está ampliamente extendido, siendo común en numerosas especies (Candolin 2003) y, sin embargo, las razones últimas para usar más de una señal no son fáciles de explicar. ¿Por qué muchas especies invierten en señales múltiples, pudiendo concentrarse sólo en una?

Existen numerosas hipótesis al respecto (Candolin 2003). Entre ellas, destacan por su interés evolutivo aquellas que proponen que las señales múltiples son caracteres adaptativos, como la hipótesis de Múltiples Mensajes (Møller & Pomiankowski 1993; Hill & McGraw 2006; Senar & Quesada 2006). Esta hipótesis propone que diferentes señales proporcionan información acerca de diferentes cualidades, que pueden ser evaluadas en conjunto o individualmente por distintos receptores.

Tradicionalmente, estas hipótesis adaptativas se han supuesto en escenarios estáticos, bajo presiones de selección constantes (Bro-Jørgensen 2010). Sin embargo, se ha propuesto la necesidad de ampliar este marco estático, para dar cabida a la plasticidad de selección resultante de la heterogeneidad ambiental (Cornwallis & Uller 2010).

Una reciente revisión sugiere que estas presiones fluctuantes del ambiente podrían tener un rol importante en el origen y mantenimiento de las señales múltiples (Bro-Jørgensen 2010). En un ambiente donde los cambios ocurren más rápido cada día, las señales múltiples podrían permitir una selección más dinámica y flexible (Bro-Jørgensen 2010). Las implicaciones evolutivas de esta hipótesis enfatizan la importancia de realizar nuevos estudios sobre señales múltiples.

El color del plumaje de las aves y su papel en la señalización múltiple

Muchas de las coloridas estructuras que podemos observar en el plumaje de las aves son señales de calidad individual, fruto de selección intersexual y competición directa entre individuos del mismo sexo (Andersson 1994; Berglund et al. 1996).

Estas señales pueden ser clasificadas según sus orígenes, los cuales a menudo también determinan las funciones de esas señales, dados los diferentes costes de producción de cada tipo de señal (Badyaev & Hill 2000; Fitze & Richner 2002; Senar et al. 2003).

Dos de los pigmentos más comunes en el plumaje de las aves son las melaninas, responsables, principalmente, de colores oscuros y pardos, y los carotenoides, que suelen ser los pigmentos encargados de colorear estructuras amarillas, rojas o anaranjadas (Hill & McGraw 2006).

Las melaninas son sintetizadas por el propio organismo, y su producción está controlada genéticamente (Buckley 1987), aunque también puede ser afectada en parte por el ambiente (Galván & Alonso-Alvarez 2009). Los animales, sin embargo, no pueden sintetizar carotenoides y han de obtenerlos a través de la dieta (Goodwin 1984).

Las diferencias entre estos pigmentos han llevado a plantear que melaninas y carotenoides podrían señalizar distintos componentes de calidad (Møller & Pomiankowski 1993; Hill & McGraw 2006; Senar & Quesada 2006). Y así, por ejemplo, mientras estructuras coloreadas por carotenoides podrían proporcionar información acerca de niveles de endoparásitos y respuesta inmune, las melaninas serían mejor reflejo de salud nutricional, ectoparasitismo y niveles hormonales relacionados con comportamientos agonísticos y competición intrasexual (Jawor & Breitwisch 2003; Hill & McGraw 2006; Quesada & Senar 2007). Esta diferenciación da soporte a la hipótesis de que múltiples señales de colores podrían señalizar diferentes cualidades del individuo a través de esos diferentes pigmentos (Candolin 2003). Sin embargo, estas diferencias han sido recientemente cuestionadas (Griffith et al. 2006), y se requieren estudios adicionales de las

posibles diferentes funciones de caracteres basados en melaninas y carotenoides, idealmente en especies que presenten de forma simultánea estos dos pigmentos.

El carbonero común (*Parus major*) y el lúgano (*Carduelis spinus*) son dos especies de aves en las que los dos tipos de pigmentos están presentes. En esta tesis se realizaron diferentes experimentos con estas aves como modelos, con el objeto de alcanzar los siguientes objetivos.

Objetivos

- Investigar si melaninas y carotenoides señalizan diferentes cualidades del individuo, permitiendo múltiples criterios en selección sexual y social (Candolin 2003). Se estudió la posible señalización de dos cualidades que pueden determinar en gran medida la respuesta a variaciones ambientales: capacidad cognitiva y personalidad.

- Examinar cómo las señales múltiples basadas en diferentes coloraciones pueden afectar el comportamiento sexual extra pareja. Este importante componente de la selección sexual es considerado un mecanismo clave a la hora de superar las consecuencias negativas de un ambiente fluctuante (Botero & Rubenstein 2012).

- Explorar cómo múltiples características de un individuo interactúan a la hora de determinar la selección de pareja. ¿Confían los animales ciegamente en las señales, o también tiene importancia su experiencia previa? La familiaridad podría tener una función potencial en la selección de pareja, siendo especialmente relevante en un ambiente variable, en el que algunas señales de calidad podrían perder fiabilidad.

Resultados, discusión y conclusiones

En el primer capítulo se examinó la función señalizadora de la banda alar amarilla del lúgano, un ornamento sexual basado en carotenoides. Para ello se midió la habilidad de diferentes machos de lúgano a la hora de resolver un problema de forrajeo y se relacionó esta habilidad con la longitud de sus bandas alares. El experimento mostró que machos con bandas alares mayores eran más rápidos resolviendo el problema. Estos resultados sugieren que podría existir selección sexual de habilidades cognitivas en el lúgano, a través de una señal basada en carotenoides.

En el segundo capítulo, estudiamos la función del babero negro del lúgano como posible fuente de información acerca de la personalidad del individuo. Para ello, examinamos la tendencia exploratoria de varios machos de lúgano, colocando un objeto novedoso en la jaula de cada ave, y midiendo el tiempo que cada individuo empleaba en acercarse a dicho objeto nuevo. Descubrimos que lúganos con baberos negros de mayor tamaño mostraban latencias de aproximación más cortas. Este hallazgo apoya la idea de que ornamentos del plumaje pueden proporcionar información acerca de diferentes rasgos de la personalidad del individuo.

Los resultados de estos dos primeros capítulos ponen de manifiesto que señales de colores, basadas en diferentes pigmentos, tienen funciones distintas en el lúgano, permitiendo un criterio múltiple en contextos de selección social y sexual (Candolin 2003).

En el tercer capítulo se examinó cómo las señales múltiples basadas en diferentes coloraciones pueden afectar el comportamiento sexual extra pareja. Para ello, evaluamos la respuesta de distintos machos de carbonero a intrusiones territoriales experimentales en sus nidos. Pudimos constatar que la respuesta agonística de los machos residentes dependía de la interacción entre la corbata negra, basada en melaninas, y la coloración amarilla, basada en carotenoides, del pecho de estos machos residentes. Aquellos machos cuyo plumaje amarillo mostraba altos niveles de saturación y, al mismo tiempo, presentaban una corbata negra de mayor tamaño, defendieron de forma menos intensa su nido que individuos que mostraban sólo altos niveles de calidad en uno de los dos ornamentos.

Tras realizar pruebas de paternidad, observamos que los machos que mostraban altos niveles de calidad en ambos tipos de ornamentos sufrían tanta infidelidad por parte de sus parejas como los individuos con ornamentos de peor calidad. Sin embargo, aquellos individuos que mostraban altos niveles de calidad en uno sólo de sus ornamentos parecían ser mucho más efectivos a la hora de mantener su paternidad intacta. Estos resultados sugieren que los machos de carbonero podrían estar siguiendo diferentes estrategias reproductivas de acuerdo con su calidad individual.

En los últimos años está adquiriendo gran importancia el estudio de la interacción entre señales que se seleccionan direccionalmente (ej. ornamentos sexuales) y otras características sin ese tipo de selección, como por ejemplo la compatibilidad o la familiaridad (Colegrave et al. 2002; Joly 2011; Thunken et al. 2012). En el último capítulo, examinamos las posibles interacciones entre ornamentos sexuales y la experiencia previa (i. e. el grado de familiaridad). Mediante experimentos de selección de pareja en lúganos, analizamos cómo ornamentos y familiaridad social interactúan para influir la selección de pareja. Los resultados mostraron la preferencia de la hembra por machos conocidos, al seleccionar entre machos familiares y desconocidos, si sus ornamentos sexuales eran similares. Al seleccionar entre un macho desconocido muy ornamentado y un macho familiar menos ornamentado, las hembras no mostraron en promedio una preferencia significativa por ninguno de los dos. Nuestros resultados sugieren que la familiaridad puede ser tan importante como los ornamentos sexuales a la hora de determinar el comportamiento de la hembra en contextos de selección sexual.

Esta Tesis, en definitiva, profundiza en el hecho de que las múltiples cualidades de un individuo, reflejadas en distintas señales de color, tienen el potencial de ser parte de múltiples estrategias válidas en selección sexual y social, permitiendo una selección más plástica. Los resultados de esta tesis apoyan la hipótesis de que la plasticidad de selección podría ser uno de los mecanismos tras el origen y mantenimiento de las señales múltiples (Bro-Jørgensen 2010).

References

Almasi, B., Jenni, L., Jenni-Eiermann, S. & Roulin, A. 2010. Regulation of stress response is heritable and functionally linked to melanin-based coloration. Journal of Evolutionary Biology, 23, 987–996.

Almasi, B., Roulin, A., Jenni-Eiermann, S. & Jenni, L. 2008. Parental investment and its sensitivity to corticosterone is linked to melanin-based coloration in barn owls. Hormones and Behavior, 54, 217–223.

Andersson, M. 1994. Sexual Selection. Princeton, NJ: Princeton University Press.

Badyaev, A. V. & Hill, G. E. 2000. Evolution of sexual dichromatism: contribution of carotenoid-versus melanin-based coloration. Biological Journal of the Linnean Society, 69, 153–172.

Balenger, S., Johnson, L. & Masters, B. 2009. Sexual selection in a socially monogamous bird: male color predicts paternity success in the mountain bluebird, *Sialia currucoides.* Behavioral Ecology and Sociobiology, 63, 403–411.

Balph, M. H. 1979. Flock stability in relation to social dominance and agonistic behavior in wintering Dark-eyed Juncos. Auk, 96, 714–722.

Barnard, C. J. & Sibly, R. M. 1981. Producers and scroungers: a general model and its application to captive flocks of House Sparrows. Animal Behaviour, 29, 543–550.

Bell, A. M. & Sih, A. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecology Letters, 10, 828–834.

Benkman, C. W. 1993. Adaptation to single resources and the evolution of crossbill (*Loxia*) diversity. Ecological Monographs, 63, 305–325.

Berglund, A., Bisazza, A. & Pilastro, A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society, 58, 385–399.

Birkhead, T. R. & Møller, A. P. 1992. Sperm Competition in Birds. London: Academic Press.

Bitton, P. P., O'Brien, E. L. & Dawson, R. D. 2007. Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor.* Animal Behaviour, 74, 1777–1784.

Björklund, M., Møller, A. P., Sundberg, J. & Westman, B. 1992. Female great tits, *Parus major*, avoid extra-pair copulation attempts. Animal Behaviour, 43, 691–693.

Björklund, M. & Westman, B. 1986. Mate-guarding in the great tit: tactics of a territorial forest-living species. Ornis Scandinavica, 99–105.

Boogert, N. J., Fawcett, T. W. & Lefebvre, L. 2011. Mate choice for cognitive traits: a review of the evidence in nonhuman vertebrates. Behavioral Ecology, 22, 447–459.

Borras, A., Cabrera, J., Colome, X., Cabrera, T. & Senar, J. C. 2010. Citril finches during the winter: patterns of distribution, the role of pines and implications for the conservation of the species. Animal Biodiversity and Conservation, 33, 89–115.

Borras, A., Cabrera, J., Colome, X., Cabrera, T. & Senar, J. C. 2011. Patterns of connectivity in the Citril finch: sympatric wintering of allopatric birds? Bird Study, 58, 257–263.

Bortolotti, G. R. 2006. Natural Selection and Coloration: Protection, Concealment, Advertisement, or Deception? In: Bird Coloration. Volume 1 Mechanisms of Production (Ed. by G. E. Hill & K. J. McGraw), pp. 3–35. Cambridge: Harvard University Press.

Botero, C. A. & Rubenstein, D. R. 2012. Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. Plos ONE, 7, e32311.

Bro-Jørgensen, J. 2010. Dynamics of multiple signalling systems: animal communication in a world in flux. Trends in Ecology & Evolution, 25, 292–300.

Brush, A. H. 1978. Avian pigmentation. In: Chemical Zoology, Vol. X, Aves (Ed. by A. H. Brush), pp. 141–161. New York: Academic Press.

Buckley, P. A. 1987. Epilogue and prologue: past and future research in avian genetics. F. Cooke and P-A. Buckley, editors. Avian genetics: a population and ecological approach. Academic Press, San Diego, 453–475.

Burtt, E. H. 1979. Tips on wings and other things. In: The Behavioral Significance of Color (Ed. by E. H. Burtt), pp. 75–110. New York: Garland STPM Press.

Burtt, E. H. 1984. Colour of the upper mandible: an adaptation to reduce reflectance. Animal Behaviour, 32, 652–658.

Burtt, E. H. 1986. An analysis of physical, and optical aspects of avian coloration with emphasis on wood-warblers. Ornithological Monographs, 38, 1–126.

Candolin, U. 2003. The use of multiple cues in mate choice. Biological Reviews, 78, 575–595.

Chappell, J. & Kacelnik, A. 2002. Tool selectivity in a non-primate, the New Caledonian crow (*Corvus moneduloides*). Animal Cognition, 5, 71–78.

Cheetham, S. A., Thom, M. D., Beynon, R. J. & Hurst, J. L. 2008. The effect of familiarity on mate choice. Chemical Signals in Vertebrates, 11, 271–280.

Choudhury, S. & Black, J. M. 1994. Barnacle geese preferentially pair with familiar associates from early-life. Animal Behaviour, 48, 81–88.

Chuang-Dobbs, H. C., Webster, M. S. & Holmes, R. T. 2001. Paternity and parental care in the black-throated blue warbler, *Dendroica caerulescens.* Animal Behaviour, 62, 83–92.

Cole, E. F. & Quinn, J. L. 2012. Personality and problem-solving performance explain competitive ability in the wild. Proceedings of the Royal Society B: Biological Sciences, 279, 1168–1175.

Colegrave, N., Kotiaho, J. S. & Tomkins, J. L. 2002. Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. Evolutionary Ecology Research, 4, 911–917.

Cooke, F. & Buckley, P. A. 1987. Avian genetics: A field and ecological approach.

Cornwallis, C. K. & Uller, T. 2010. Towards an evolutionary ecology of sexual traits. Trends in Ecology & Evolution, 25, 145–152.

Cott, H. B. 1940. Adaptive coloration in animals.

Cristol, D. A. 1995. The coat-tail effect in merged flocks of dark-eyed juncos: social status depends on familiarity. Animal Behaviour, 50, 151–159.

Dale, J. 2006. Intraspecific variation in bird colors. In: Bird Coloration, Volume 2, Function and Evolution (Ed. by G. E. Hill & K. J. McGraw) Cambrigde, MA: Harvard University Press.

Danchin, E., Giraldeau, L. A., Valone, T. J. & Wagner, R. H. 2004. Public information: from nosy neighbors to cultural evolution. Science, 305, 487–491.

Darwin, C. 1871. The Descent of Man and Selection in Relation to Sex. London: John Murray.

David, M., Cézilly, F. & Giraldeau, L. A. 2011. Personality affects zebra finch feeding success in a producer-scrounger game. Animal Behaviour, 82, 61–67.

Dingemanse, N. J., Both, C., Drent, P. J. & Tinbergen, J. M. 2004. Fitness consequences of avian personalities in a fluctuating environment. Proceedings of the Royal Society B: Biological Sciences, 271, 847–852.

Dingemanse, N. J. & de Goede, P. 2004. The relation between dominance and exploratory behavior is context-dependent in wild great tits. Behavioral Ecology, 15, 1023–1030.

Dingemanse, N. J. & Réale, D. 2005. Natural selection and animal personality. Behaviour, 142, 1159–1184.

Drent, P. J., van Oers, K. & van Noordwijk, A. J. 2003. Realized heritability of personalities in the great tit (*Parus major*). Proceedings of the Royal Society B: Biological Sciences, 270, 45–51.

Ducrest, A., Keller, L. & Roulin, A. 2008. Pleiotropy in the melanocortin system colouration and behavioural syndromes. Trends in Ecology and Evolution, 23, 502–510.

Edelaar, P., Siepielski, A. M. & Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. Evolution, 62, 2462–2472.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution, 34, 76–91.

Exnerová, A., Svádová, K. H., Fucíková, E., Drent, P. & Štys, P. 2010. Personality matters: individual variation in reactions of naive bird predators to aposematic prey. Proceedings of the Royal Society B: Biological Sciences, 277, 723–728.

Ferns, P. N. & Hinsley, S. A. 2008. Carotenoid plumage hue and chroma signal different aspects of individual and habitat quality in tits. Ibis, 150, 152–159.

Figuerola, J. & Senar, J. C. 2005. Seasonal changes in carotenoid- and melanin-based plumage coloration in the Great Tit *Parus major*. Ibis, 147, 797–802.

Fisher, J. & Hinde, R. A. 1949. The opening of milk bottles by birds. British Birds, 42, 347–357.

Fitze, P. S. & Richner, H. 2002. Differential effects of a parasite on ornamental structures based on melanins and carotenoids. Behavioral Ecology, 13, 401–407.

Forschler, M. I., Senar, J. C., Borras, A., Cabrera, J. & Björklund, M. 2011. Gene flow and range expansion in a mountain-dwelling passerine with a fragmented distribution. Biological Journal of the Linnean Society, 103, 707–721.

Forstmeier, W. 2007. Do individual females differ intrinsically in their propensity to engage in extra-pair copulations? Plos ONE, 2, e952.

Galván, I. & Alonso-Alvarez, C. 2008. An intracellular antioxidant determines the expression of a melanin-based signal in a bird. Plos ONE, 3, e3335.

Galván, I. & Alonso-Alvarez, C. 2009. The expression of melanin-based plumage is separately modulated by exogenous oxidative stress and a melanocortin. Proceedings of the Royal Society B: Biological Sciences, 276, 3089–3097.

Garamszegi, L. Z., Eens, M. & Török, J. 2008. Birds reveal their personality when singing. Plos ONE, 3, e2647.

Gavish, L. & Gavish, B. 1981. Patterns that conceal a bird's eye. Zeitschrift für Tierpsychologie, 56, 193–204.

Glück, E. 1980. Ernährung und Nahrungsstrategie des Stieglitzes Carduelis carduelis. Ökologie der Vögel (Ecology of Birds), 2, 43–91.

Godin, J. G. & Dugatkin, L. A. 1996. Female mating preference for bold males in the guppy, *Poecilia reticulata*. Proceedings of the National Academy of Sciences, 93, 10262–10267.

Goodwin, T. W. 1984. The Biochemistry of the Carotenoids. Chapman and Hall New York.

Gosler, A. G. 1993. The Great Tit. London: Hamlyn.

Grether, G. F. 2000. Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). Evolution, 54, 1712–1724.

Grether, G. F., Hudon, J. & Millie, D. F. 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. Proceedings of the Royal Society B: Biological Sciences, 266, 1317–1322.

Griffith, S. C., Parker, T. H. & Olson, V. A. 2006. Melanin- versus carotenoid-based sexual signals: is the difference really so black and red? Animal Behaviour, 71, 749–763.

Groothuis, T. G. G. & Carere, C. 2005. Avian personalities: characterization and epigenesis. Neuroscience and Biobehavioral Reviews, 29, 137–150.

Groth, J. G. 1993. Call matching and positive assortative mating in Red Crossbills. Auk, 110, 398–401.

Hansen, B. T., Johannessen, L. E. & Slagsvold, T. 2009. Interspecific cross-fostering affects mate guarding behaviour in great tits (*Parus major*). Behaviour, 146, 1349–1361.

Håstad, O., Victorsson, J. & Ödeen, A. 2005. Differences in color vision make passerines less conspicuous in the eyes of their predators. Proceedings of the National Academy of Sciences, 102, 6391–6394.

Hearing, V. J. 1993. Unraveling the melanocyte. American Journal of Human Genetics, 52, 1–7.

Hill, G. E. 2006. Environmental Regulation of Ornamental Coloration. In: Bird Coloration, Volume 1, Mechanisms and Measurements (Ed. by G. E. Hill & K. J. McGraw), pp. 507–560. Cambrigde, MA: Harvard University Press.

Hill, G. E. & McGraw, K. J. 2006. Bird Coloration. Volume 2. Function and Evolution. Cambridge: Harvard University Press.

Houde, A. E. 1997. Sex, Color, and Mate Choice in Guppies. Princeton, NJ: Princeton University Press.

Huntingford, F. A. 1976. The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Animal Behaviour, 24, 245–260.

Järvi, T. & Bakken, M. 1984. The function of the variation in the breast stripe of the Great tit (*Parus major*). Animal Behaviour, 32, 590–596.

Jawor, J. M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. Auk, 120, 249–265.

Johnsen, A. & Lifjeld, J. T. 1995. Unattractive males guard their mates more closely: An experiment with bluethroats (Aves, Turdidae: *Luscinid-s-suecica*). Ethology, 101, 200–212.

Joly, E. 2011. The existence of species rests on a metastable equilibrium between inbreeding and outbreeding. An essay on the close relationship between speciation, inbreeding and recessive mutations. Biology Direct, 6.

Jones, K. & Godin, J. G. 2010. Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. Proceedings of the Royal Society B: Biological Sciences, 277, 625–632.

Karino, K., Shinjo, S. & Sato, A. 2007. Algal-searching ability in laboratory experiments reflects orange spot coloration of the male guppy in the wild. Behaviour, 144, 101–113.

Keagy, J., Savard, J. F. & Borgia, G. 2009. Male satin bowerbird problem-solving ability predicts mating success. Animal Behaviour, 78, 809–817.

Kempenaers, B. 1995. Polygyny in the blue tit: Intra- and inter-sexual conflicts. Animal Behaviour, 49, 1047–1064.

Kempenaers, B., Verheyen, G. R. & Dhondt, A. A. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behavioral Ecology, 8, 481–492.

Kempenaers, B., Verheyen, G. R., van den Broeck, M., Burke, T., van Broeckhoven, C. & Dhondt, A. A. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. Nature, 357, 494–496.

Kittilsen, S., Schjoulden, J., Beitnes-Johansen, I., Shaw, J. C., Pottinger, T. G., Sorensen, C., Braastad, B. O., Bakken, M. & Overli, O. 2009. Melanin-based skin spots reflect stress responsiveness in salmonid fish. Hormones and Behaviour, 56, 292–298.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behavioral Ecology and Sociobiology, 25, 393–401.

Kokko, H. & Morrell, L. J. 2005. Mate guarding, male attractiveness, and paternity under social monogamy. Behavioral Ecology, 16, 724–731.

Kurvers, R. H. J. M., Prins, H. H. T., van Wieren, S. E., van Oers, K., Nolet, B. A. & Ydenberg, R. C. 2010a. The effect of personality on social foraging: shy barnacle geese scrounge more. Proceedings of the Royal Society B: Biological Sciences, 277, 601–608.

Kurvers, R. H. J. M., van Oers, K., Nolet, B. A., Jonker, R. M., van Wieren, S. E., Prins, H. H. T. & Ydenberg, R. C. 2010b. Personality predicts the use of social information. Ecology Letters, 13, 829–837.

Lefebvre, L. 2000. Feeding innovations and their cultural transmission in bird populations. In: The Evolution of Cognition (Ed. by C. Heyes & L. Huber), pp. 311–328. Cambridge: MIT Press.

Lubjuhn, T. 1995. Reproductive strategies and parental effort in birds: great tits (*Parus major*) - a case study. Verhandlungen der Deutschen Zoologischen Gesellschaft, 88, 15–21.

Lubjuhn, T., Curio, E., Muth, S. C., Brün, J. & Epplen, J. T. 1993. Influence of extra-pair paternity on parental care in great tits (*Parus major*). In: DNA Fingerprinting: State of the Science (Ed. by S. D. J. Pena, R. Chakraborty, J. T. Epplen & A. J. Jeffreys), pp. 379–385. Basel/Switzerland: Birkhäuser Verlag.

Mace, R. 1987. The dawn chorus in the great tit paras major is directly related to female fertility. Nature, 330, 745–746.

Mafli, A., Wakamatsu, K. & Roulin, A. 2011. Melanin-based coloration predicts aggressiveness and boldness in captive eastern Hermann's tortoises. Animal Behaviour, 81, 859–863.

McGraw, K. J. 2006. Mechanics of Melanin-based Coloration. In: Bird Coloration, Volume 1, Mechanisms and Measurements (Ed. by G. E. Hill & K. J. McGraw) Cambrigde, MA: Harvard University Press.

McGraw, K. J. 2008. An update on the honesty of melanin-based color signals in birds. Pigment Cell and Melanoma Research, 21, 133–138.

McGraw, K. J. & Ardia, D. R. 2007. Do carotenoids buffer testosterone-induced immunosuppression? An experimental test in a colourful songbird. Biology Letters, 3, 375–378.

McGraw, K. J., Beebee, M. D., Hill, G. E. & Parker, R. S. 2003. Lutein-based plumage coloration in songbirds is a consequence of selective pigment incorporation into feathers. Comparative Biochemistry and Physiology Part B, 135, 689–696.

McGraw, K. J. & Hill, G. E. 2000a. Carotenoid-based ornamentation and status signaling in the house finch. Behavioral Ecology, 11, 520–527.

McGraw, K. J. & Hill, G. E. 2000b. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proceedings of the Royal Society B: Biological Sciences, 267, 1525–1531.

McGraw, K. J., Medina-Jerez, W. & Adams, H. 2007. Carotenoid-based plumage coloration and aggression during molt in male house finches. Behaviour, 144, 165–178.

Møller, A. P. & Birkhead, T. R. 1994. The evolution of plumage brightness in birds is related to extrapair paternity. Evolution, 48, 1089–1100.

Møller, A. P. & Pomiankowski, A. 1993. Why have birds got multiple sexual ornaments? Behavioral Ecology and Sociobiology, 32, 167–176.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist, 139, 971–989.

Mundinger, P. C. 1979. Call learning in the carduelinae: ethological and systematic considerations. Systematic Zoology, 28, 270–283.

Negro, J. J., Sarasola, J. H., Fariñas, F. & Zorrilla, I. 2006. Function and occurrence of facial flushing in birds. Comparative Biochemistry and Physiology-Part A: Molecular and Integrative Physiology, 143, 78–84.

Newton, I. 1972. Finches. London: Collins.

Newton, I. 2008. The Migration Ecology of Birds. London: Academic Press.

O'Brien, E. L. & Dawson, R. D. 2010. Plumage color and food availability affect male reproductive success in a socially monogamous bird. Behavioral Ecology, 22, 66–72.

Parchman, T. L., Benkman, C. W. & Britch, S. C. 2006. Patterns of genetic variation in the adaptive radiation of New World crossbills (Aves: *Loxia*). Molecular Ecology, 15, 1873–1887.

Patricelli, G. L., Uy, J. A. C. & Borgia, G. 2003. Multiple male traits interact: attractive bower decorations facilitate attractive behavioural displays in satin bowerbirds. Proceedings of the Royal Society B: Biological Sciences, 270, 2389–2395.

Patrick, S. C., Chapman, J. R., Dugdale, H. L., Quinn, J. L. & Sheldon, B. C. 2011. Promiscuity, paternity and personality in the great tit. Proceedings of the Royal Society B: Biological Sciences, 279, 1724–1730.

Price, T. D., Yeh, P. J. & Harr, B. 2008. Phenotypic plasticity and the evolution of a socially selected trait following colonization of a novel environment. American Naturalist, 172, S49–S62.

Prota, G. 1995. The chemistry of melanins and melanogenesis. Fortschritte der Chemie organischer Naturstoffe, 64, 93–148.

Prum, R. O. 2006. Anatomy, physics, and evolution of structural colors. In: Bird Coloration: Volume 1. Mechanisms and Measurements (Ed. by G. E. Hill & K. J. McGraw), pp. 295–353. Cambridge: Harvard University Press.

Quesada, J. & Senar, J. C. 2007. The role of melanin- and carotenoid-based plumage coloration in nest defence in the Great Tit. Ethology, 113, 640–647.

Ratcliffe, J. M., Fenton, M. B. & Shettleworth, S. J. 2006. Behavioral flexibility positively correlated with relative brain volume in predatory bats. Brain, Behavior and Evolution, 67, 165–176.

Reader, S. M. & Laland, K. N. 2002. Social intelligence, innovation, and enhanced brain size in primates. Proceedings of the National Academy of Sciences, 99, 4436–4441.

Réale, D, Dingemanse, N. J., Kazem, A. J. N. & Wright, J. 2010. Evolutionary and ecological approaches to the study of personality. Philosophical Transactions of the Royal Society B: Biological Sciences, 365, 3937–3946.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews, 82, 291–318.

Ripoll, J., Saldaña, J. & Senar, J. C. 2004. Evolutionary stable transition rates in a stage-structured model. An application to the analysis of size distributions of badges of social status. Mathematical Biosciences, 190, 145–181.

Rivera-Gutierrez, H. F., Pinxten, R. & Eens, M. 2010. Multiple signals for multiple messages: great tit, *Parus major*, song signals age and survival. Animal Behaviour, 80, 451–459.

Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. Biological Reviews, 79, 1–34.

Roulin, A., Gasparini, J., Bize, P., Ritschard, M. & Richner, H. 2008. Melanin-based colorations signal strategies to cope with poor and rich environments. Behavioral Ecology and Sociobiology, 62, 507–519.

Roulin, A., Jungi, T. W., Pfister, H. & Dijkstra, C. 2000. Female barn owls (*Tyto alba*) advertise good genes. Proceedings of the Royal Society B: Biological Sciences, 267, 937–941.

Sanz, J. J. 2001. Experimentally reduced male attractiveness increases parental care in the pied flycatcher *Ficedula hypoleuca*. Behavioral Ecology, 12, 171–176.

Schluter, D., Boughman, J. W. & Rundle, H. D. 2001. Parallel speciation with allopatry. Trends in Ecology and Evolution, 16, 283–284.

Schuett, W. & Dall, S. R. X. 2009. Sex differences, social context and personality in zebra finches, *Taeniopygia guttata*. Animal Behaviour, 77, 1041–1050.

Schuett, W., Tregenza, T. & Dall, S. R. X. 2010. Sexual selection and animal personality. Biological Reviews, 85, 217–246.

Senar, J. C. 1985. Interactional rules in captive Siskins (*Carduelis spinus*). Miscel·lània Zoològica, 9, 347–360.

Senar, J. C. 2006. Color Displays as Intrasexual Signals of Aggression and Dominance. In: Bird Coloration, Volume 2: Function and Evolution (Ed. by G. E. Hill & K. J. McGraw) Cambridge, MA.: Harvard University Press.

Senar, J. C., Borras, A., Cabrera, J., Cabrera, A. & Björklund, M. 2006. Local differentiation in the presence of gene flow in the citril finch *Serinus citrinella*. Biology Letters, 2, 85–87.

Senar, J. C., Burton, P. J. K. & Metcalfe, N. B. 1992. Variation in the nomadic tendency of a wintering finch *Carduelis spinus* and its relationship with body condition. Ornis Scandinavica, 23, 63–72.

Senar, J. C. & Camerino, M. 1998. Status signalling and the ability to recognize dominants: an experiment with siskins (*Carduelis spinus*). Proceedings of the Royal Society B: Biological Sciences, 265, 1515–1520.

Senar, J. C., Camerino, M., Copete, J. L. & Metcalfe, N. B. 1993. Variation in black bib of the Eurasian Siskin (*Carduelis spinus*) and its role as a reliable badge of dominance. Auk, 110, 924–927.

Senar, J. C., Camerino, M. & Metcalfe, N. B. 1989. Agonistic interactions in siskin flocks: why are dominants sometimes subordinate? Behavioral Ecology and Sociobiology, 25, 141–145.

Senar, J. C., Camerino, M. & Metcalfe, N. B. 1990. Familiarity breeds tolerance: the development of social stability in flocking Siskins (*Carduelis spinus*). Ethology, 85, 13–24.

Senar, J. C., Camerino, M. & Metcalfe, N. B. 1997. A comparison of agonistic behaviour in two Cardueline finches: feudal species are more tolerant than despotic ones. Etología, 5, 73–82.

Senar, J. C., Camerino, M. & Uribe, F. 2001. Body mass regulation in resident and transient wintering siskins *Carduelis spinus*. Etología, 9, 47–52.

Senar, J. C., Copete, J. L. & Metcalfe, N. B. 1990. Dominance relationships between resident and transient wintering Siskins. Ornis Scandinavica, 21, 129–132.

Senar, J. C. & Domènech, J. 2011. Sex-specific aggression and sex ratio in wintering finch flocks: serins and siskins differ. Acta Ethologica, 14, 7–11.

Senar, J. C., Domènech, J. & Camerino, M. 2005. Female Siskins choose mate by the size of the yellow wing stripe. Behavioral Ecology and Sociobiology, 57, 445–469.

Senar, J. C., Figuerola, J. & Domènech, J. 2003. Plumage coloration and nutritional condition in the Great tit *Parus major*: the roles of carotenoids and melanins differ. Naturwissenschaften, 90, 234–237.

Senar, J. C., Lleonart, J. & Metcalfe, N. B. 1994. Wing-shape variation between resident and transient wintering Siskins *Carduelis spinus*. Journal of Avian Biology, 25, 50–54.

Senar, J. C., Negro, J. J., Quesada, J., Ruiz, I. & Garrido, J. 2008. Two pieces of information in a single trait?: the yellow breast of the Great Tit reflects both pigment acquisition and body condition. Behaviour, 145, 1195–1210.

Senar, J. C., Polo, V., Uribe, F. & Camerino, M. 2000. Status signalling, metabolic rate and body mass in the siskin: the cost of being a subordinate. Animal Behaviour, 59, 103–110.

Senar, J. C. & Quesada, J. 2006. Absolute and relative signals: a comparison between melanin- and carotenoid-based patches. Behaviour, 143, 589–595.

Sheldon, B. C. & Ellegren, H. 1999. Sexual selection resulting from extrapair paternity in collared flycatchers. Animal Behaviour, 57, 285–298.

Sherman, P. W. & Morton, M. L. 1988. Extra-pair fertilizations in mountain white-crowned sparrows. Behavioral Ecology and Sociobiology, 22, 413–420.

Sih, A., Bell, A. & Johnson, J. C. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution (Personal edition), 19, 372–378.

Slagsvold, T. 1993. Sex recognition and breast stripe size in great tits. Ardea, 81, 35–42.

Smith, B. R. & Blumstein, D. T. 2007. Fitness consequences of personality: a meta-analysis. Behavioral Ecology, 19, 448–455.

Snowberg, L. K. & Benkman, C. W. 2007. The role of marker traits in the assortative mating within red crossbills, *Loxia curvirostra* complex. Journal Evolutionary Biology, 20, 1924–1932.

Snowberg, L. K. & Benkman, C. W. 2009. Mate choice based on a key ecological performance trait. Journal of Evolutionary Biology, 22, 762–769.

Sol, D., Duncan, R. P., Blackburn, T. M., Cassey, P. & Lefebvre, L. 2005. Big brains, enhanced cognition, and response of birds to novel environments. Proceedings of the National Academy of Sciences, 102, 5460–5465.

Sol, D., Szekely, T., Liker, A. & Lefebvre, L. 2007. Big-brained birds survive better in nature. Proceedings of the Royal Society B: Biological Sciences, 274, 763–769.

Sol, D., Timmermans, S. & Lefebvre, L. 2002. Behavioural flexibility and invasion success in birds. Animal Behaviour, 63, 495–502.

Stradi, R. 1998. The Colour of Flight: Carotenoids in Bird Plumage. Milano: Solei Press.

Summers, R. W., Dawson, R. J. G. & Phillips, R. E. 2007. Assortative mating and patterns of inheritance indicate that the three crossbill taxa in Scotland are species. Journal of Avian Biology, 38, 153–162.

Svensson, L. 1975. Identification Guide to European Passerines. 2nd ed. Stockholm: Naturhistoriska Riksmuseet.

Tanaka, Y. 1996. Social selection and the evolution of animal signals. Evolution, 50, 512–523.

Thunken, T., Meuthen, D., Bakker, T. C. M. & Baldauf, S. A. 2012. A sex-specific trade-off between mating preferences for genetic compatibility and body size in a cichlid fish with mutual mate choice. Proceedings of the Royal Society B: Biological Sciences.

Titulaer, M., van Oers, K. & Naguib, M. 2012. Personality affects learning performance in difficult tasks in a sex-dependent way. Animal Behaviour, 83, 723–730.

Toral, G. M., Figuerola, J. & Negro, J. J. 2008. Multiple ways to become red: pigment identification in red feathers using spectrometry. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 150, 147–152.

van den Brink, V., Dolivo, V., Falourd, X., Dreiss, A. N. & Roulin, A. 2011. Melanic color-dependent antipredator behavior strategies in barn owl nestlings. Behavioral Ecology, 23, 473–480.

van Oers, K., Drent, P. J., Dingemanse, N. J. & Kempenaers, B. 2008. Personality is associated with extrapair paternity in great tits, *Parus major*. Animal Behaviour, 76, 555–563.

van Oers, K., Drent, P. J., Goede, P. & van Noordwijk, A. J. 2004. Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. Proceedings of the Royal Society B: Biological Sciences, 271, 65–73.

van Overveld, T. & Matthysen, E. 2010. Personality predicts spatial responses to food manipulations in free-ranging great tits (*Parus major*). Biology Letters, 6, 187–190.

Verbeek, M. E. M., Drent, P. J. & Wiepkema, P. R. 1994. Consistent individual differences in early exploratory behaviour of male great tits. Animal Behaviour, 48, 1113–1121.

Wallace, A. R. 1889. Darwinism. London: Macmillan.

West, P. M. & Packer, C. 2002. Sexual selection, temperature, and the lion's mane. Science, 297, 1339–1343.

Westneat, D. F. 1987. Extra-pair fertilizations in a predominantly monogamous bird: genetic evidence. Animal Behaviour, 35, 877–886.

Wheeler, B. K. 2003. Raptors of Western North America: The Wheeler Guides. Princeton University Press.

Wilson, W. H. J. 2006. Evidence of flock fidelity in Common Redpolls and Pine Siskins. North American Bird Bander, 31, 105–109.

Wolf, B. O. & Walsberg, G. E. 2000. The role of the plumage in heat transfer processes of birds. American Zoologist, 40, 575–584.

Zajitschek, S. R. K. & Brooks, R. C. 2008. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). American Naturalist, 172, 843–854.

Zajitschek, S. R. K., Evans, J. P. & Brooks, R. 2006. Independent effects of familiarity and mating preferences for ornamental traits on mating decisions in guppies. Behavioral Ecology, 17, 911–916.

Acknowledgments

Acknowledgments

Cierto, ¡la Ciencia es dura! Esta tesis me ha hecho perder sueño, salud, pelo... en resumen, una parte significativa de mi juventud. Bueno, tampoco es para tanto... decir "la tesis" es quizá demasiada generalización. Básicamente los culpables de mi incipiente alopecia fueron el rotavapor, los patrones de isótopos estables y el Reference Manager. Quitando esos artefactos infernales, el resto de este viaje ha sido una experiencia magnífica, y llega el momento de agradecerla a quienes la hicieron posible.

El primero ha de ser, obviamente, mi director. Anda que no lo hemos pasado bien, ¿eh JC? Trabajar contigo ha sido sugestivo, estimulante y, a veces, todo un reto! (Me tuve que ir a América para poder acostarme más tarde que tú. Y ni aún así lo conseguía...)

Tengo mucho que agradecerte: Comenzaste ofreciéndome una oportunidad. Después me entregaste tu tiempo, tu experiencia, geniales ideas, todos los medios e incluso algunos trucos. Gracias a ti aprendí –seguimos trabajando en ello- a moldear mi procrastinación into multitasking. Me presentaste a sobresalientes científicos y me diste la oportunidad de conocer a personas incluso más importantes (aunque por esa regla de tres, también es culpa tuya que ahora viva en un país húmedo y frío...).

Por último, me enorgullece poder decir que me otorgaste tu amistad. Poco a poco (y, especialmente desde aquellos días bregando juntos contra el R en Canterbury!) he ido conociendo a un camarada en el que puedo confiar. Joan Carles, ha sido un honor estar juntos en todas estas batallas. Y espero que aún otras tantas venideras nos reúnan bajo el mismo estandarte.

No sé cómo te convenció, pero estoy seguro de que debo agradecerle a Tinín un montón de exageradas recomendaciones. Para corresponder a ese inmenso favor, y a bastantes más, debo dar un salto atrás y volver a **Badajoz** (y así escribo esto con algo de orden).

Corría el año 1999 cuando este tierno y bisoño estudiante de biología se presentaba, pleno de determinación e inocencia, en el Departamento de Zoología de la UEx. Yo me aburría soberanamente en clase, y lo quería realmente era "ser etólogo", aunque no tuviera muy claro en qué consistía aquello. Así que subí allí donde supuse estarían "los etólogos" y pregunté. Alguien me indicó: "mira a ver allí, al fondo del pasillo". Quiso la suerte que aquel fuera el despacho de Tinín. "Quiero trabajar en etología", le dije. Un rato después, ya estaba yo en el aviario, henchido de orgullo, llenando comederos de alpiste y cambiando bebederos.

El resto de la carrera lo hice subido a una escalera a la que llamábamos Penélope (era nuestra Cruz), y la pizarra la veía desde las ventanas, mientras anillaba pollos de gorrión. Pero creo que lo que aprendí sobre aquella escalera compensó con creces lo que pudiera haberme perdido por no ir a clase (que no fue mucho, gracias a los magníficos apuntes de Marga y la ayuda extraterrestre de Fernando Mayo). Tinín me abrió las puertas a la ciencia, me llevó a mis primeros congresos (donde conocí a JC!) y también, de formas, a veces, digamos... originales, me presentó a algún ilustre científico.

Además de Tinín, mis primeros maestros de ornitología fueron Alfonso Marzal y Carlos Navarro y con cada uno aprendí a trabajar con mis primeras especies: golondrinas, aviones y gorriones. Nacho (a.k.a. "Gar") recorrió exactamente el mismo camino. De hecho, llevamos haciendo prácticamente lo mismo desde séptimo de EGB, aunque él siempre saca mejores notas :P Compartíamos a Penélope y la gente nos confundía por el campus: Éramos como Epi y Blas, pero en hetero. Con Mami Fátima tuvimos nuestro primer trabajo de biólogos licenciados. Montse me hizo feliz y se unió al club de los pajaritos, pero nunca cambió del todo sus amados gorilas por las plumas. Con Paco Ferri, fundamos "Cormorán y los tres mosqueteros", gran equipo ornitológico! Lorenzo, mi maestro de jazz, Maribel, Mario, Bettina, Fernando, Paconan, Paco Zulú, Anabel, Mariajo y el resto de compañeros de facultad, departamento y prácticas, ahí va mi recuerdo.

En Badajoz conocí a mis amigos rancios, los de siempre. El Club de la Alegría ha sido

uno de mis mayores apoyos durante todos estos años ("mira que la fecha de lectura de tesis no coincida con un Ironman") y, englobando al anterior y llenándolo de adorables risas femeninas, los Chichis Híbridos (ya se te podría haber ocurrido otro nombre, Luisete...). Juanma, Marti, Bree, Carlos Jerez, Beni, Germán y el resto de maristas... Paco, gracias por enseñarme a escalar!

Justo antes de marcharme, conocí a Lidia, y nos apoyamos y seguimos mutuamente por el mundo un tiempo, hasta que las presiones ecológicas fluctuantes (Bro-Jørgensen 2012) nos cambiaron irremediablemente.

Es bueno tomar perspectiva y probar otros pastos antes de embarcarte en un proyecto tan gordo como una tesis. En Logroño trabajé casi dos años para el brazo ejecutivo de la conservación, y gané mucho más que perspectiva. En la Consejería de Medio Ambiente de La Rioja encontré un bagaje formidable: Mi tutor Luis (abrazo de oso!), Nacho, Ignacio, Diego, Asun, mis compis y sufridoras de bazofia Sonia, Pilar, Soraya y Pedro (cooompañero!), Natalia (¡Ángela María!), Anabel, Vero, Ramón, las Ésteres!, Juanpa, Noa, Juanjo (a ver si consigo organizar septiembre y nos vemos en la boda!), Guille, Chus, la Fieri, Mónica, María, María M, María F, Romina, Xalo, Ana, Marta, las Teresitas, Patricia, Carmen, Miguel, Elena, Naiara y muchos más que me dejo... Chicos, todavía me emociono con el álbum! Vosotros estuvisteis ahí, tanto en los peores momentos, como en memorables escapadas por la calle Laurel (In vino veritas!). Os mando, cómo no, un abrazo.

Y con este equipaje, me fui a **Barcelona** y me puse a buscar hogar. Ari y Jordi (voy a tener un hermanito!) me recogieron de la calle cuando llegué. Me salvaron de la mendicidad, pero su ducha por poco me mata. Unos días después, me mudé a un edificio más grande, con torres y almenas, y comencé la tesis.Una de las mayores ventajas de trabajar en el **Museu de Ciències Naturals** es señalar el edificio desde la calle y poder decir "Ése es mi castillo". Sin embargo (afortunadamente), muchas otras personas compartían conmigo la propiedad del Castell dels Tres Dragons. Mi primera compañera de mazmorra fue mi helmanita Esther, que me llevaba a comer al parque y hacía que me partiese de risa con sus exabruptos (y sigue haciéndolo en cada email!). Maese Llanillo, el Dr. Carrillo y yo formábamos un formidable trío calavera durante la noche, y arreglábamos el mundo al día siguiente durante nuestras tertulias de sobremesa en el Patio Pristinal. Es una pena que Javi, el helmanito mayor, tardase tanto en volver al Museu y no hayamos coincidido en las mazmorras. Aún así, tuvimos nuestros miércoles de cerveza y (mirar) mujeres con el Albert (gracias por ayudarme con las fotos!), Julien, Ceci, el Brro... Annnnnita, duquesa de Can Catà, compartió conmigo las primeras carreras por la Carena y los mosquitos tigre de St. Quirze, por suerte Montse Camerino nos dejaba también bañarnos en la piscina (Otto! Los pantalones!). Marga se convirtió en la digna sucesora de la Duquesa, moviéndose con gracia felina tanto por Pandora como por largas listas de referencias. Ahora volverá locos a los gringos con su voz sexi de walkie-talkie. Alba "Árbit" es una heroína: Me salvó de un seguro shock anafiláctico al hacerse cargo de los luganitos de la torre. ¡Rayos y truenos! ¡Llegó a salvar incluso a uno de los lúganos, a base de boca-pico! Irene cambió los carboneros por gallinitas, y ahora es una atareada científica granjera. Muchos otros valientes se adentraron también en el bosque: Dani, Clara, Jana, Rubén, Ramón, Vanessa, Sandra, Montse, Jason, Miguel... El récord de velocidad subiendo por la Carena lo tenía Francesc Uribe, fénix de los ingenios. Mami Lluisa nos escuchaba, ponía orden en nuestros datos de campo, y sufría estoicamente la única característica de doctor que yo llevaba de serie desde el primer año: mi horrible caligrafía. Sepand, Emilio, y Josep están ahora al frente de la mazmorra y continúan la saga cancatársica con grandes gestas.

Junto a la mazmorra, las demás dependencias del castillo colaboran en el sostenimiento de la corte. Alicia era la princesa más princesa del castillo, y jugábamos a trovadores con Gemma y Amadeu. Meritxell, Vicente y Ascensi hicieron que mis usualmente infructuosas batallas con

la burocracia fueran mucho más llevaderas. La selecta lista de correo de Vicente es un remedio eficaz contra la depresión. Carles Orta (artista!) y Manuel me prestaban herramientas para el trabajo y Alex música y té para el espíritu. Pere, Jordi, Teresa, Carles, Eulalia, Gloria, Ingrid, Albert, Eusebio, Olga, Anna... Los que me dejo, los que se fueron y los que vendrán: el Museu estará siempre en mi recuerdo.

La primera estancia fuera del museu fue para estudiar isótopos y heavy metals en la **UB**. Javi Cotín fue mi maestro, algo irresponsable, al dejarme manipular nitrógeno líquido y otros venenos. Pili me salvó de cortarme las venas en la balanza jedi de precisión.

Con el **máster de Biodiversidad** en la UB, apareció un gran grupo de amigos, y dio comienzo lo que Manolo bautizó como el verano del amor (déjalo-ya...). Clavitoo (frrr frrr), Cristinita, Silvia, Raquel, el helmanito Owen (que me enseñó a bucear), Juan Pablo, Karina, Laia, Carlos, Sergi Taboada, Kriskitine (mi sucesora en Logroño!), Anna, Humberto, Iguazel, Nurita (que también me enseñó a bucear, pero ella me llevó de la mano), Oriol, Blanca, Edgar, Mary, Andrés, Adolfo... También encontré un tío con barbas que en poco tiempo se convertiría en el meu germà (y gracias a su coche pude terminar más de una temporada de campo!). A través de él apareció un montón de espíritus outsiders (y luego hallamos muchos más) Pere Bach, Pere Neanderthal, Isaac, Berni, el Geri, Javi (y las noches de Luz), Raquel, Dani, Edu, Mari Carmen (faro y guía en esta fase final de tesis), Teniente Tello, Andrea, la prima Laura, Oriol, Oleguer, David Potter, Josep, Victor, Estela, mi compix Yoel Anda, los Szganitos!, David , Cris Surfer, nuestro Dj Agus... Integrantes todos del Team Mallorca 81, bien sabéis lo importantes que sois para mí. Entre todos vosotros, habéis conseguido que estos años en Barcelona hayan sido como vivir en un anuncio de Estrella Damm (bueno, Voll-Damm, en nuestro caso). ¡Gracias!

La siguiente estancia, ya fuera de Barcelona fue en **Sevilla**, en la **Estación Biológica de Doñana**, primero en el Pabellón del Perú y luego ya en la Cartuja. Juanjo Negro, elegante anfitrión donde los haya, me ha acogido ya tantas veces que he perdido la cuenta. Junto a él, Jaime Potti, Jordi Figuerola, Juli Broggi y Pim Edelaar me aconsejaron y ayudaron con favores variopintos.Las damas del laboratorio de Ecología Química me enseñaron, con mucha paciencia, a jugar con cacharritos muy caros. A última hora de la tarde, mis kelis preferidas entraban en el laboratorio, yo cambiaba la música clásica por Pitbull y se liaba parda. Luego, como en algún sitio había que cenar, me unía a la crème de la crème de la biología internacional: Lety (te debo un masaje!), Marian, Alex (yo también tendré que llamar a los agradecimientos "capítulo 5"), Maria Viota, Alberto, Andrea, Claudia, Jorge, Clara, Josué, Marcello y Luisiño (Caballeros, es un honor contar con su amistad. No puedo esperar más para nuestra siguiente (in)gesta. O sí: "total, qué prisa tengo..."), Giulia, Maritxu, Monica Garcia, Nuno, Joaquim, Lidia, Carlos Rouco, Ana la gallega, Marga (la sevillana más auténtica que existe), Irene, Ainara (gracias por tu apoyo logístico en la última maratón de tesis!), Duarte, Laurita, Grego (y Oswell y el canijo!), Rosa, Néstor Mara, Roger, Andrea, ZuUuu! (Gracias por innumerables favores, por "nunca estar realmente busy" para mí en el gmail y, sobre todo, por cuatro años de divertida, sarcástica y leal amistad), Ana Montero, Mon Ica, Gema, Jesús, Simone (gracias por llevarme a pescar moritos!), Airam (gracias por llevarme a anillar primillas!), David, Lazslo, Mireita, todos los compis del "despacho" de la biblioteca, los Madrid-Barça con Jordi, Violeta(sueca!) y montones de guiris, Camille, Christophe "Franchute", y todos los compis de la teniente O'Laly's group, que me mantuvieron en forma y me ayudaron a controlar el estrés de la última etapa.

In 2009, after suffering the typical 40°C summer of Sevilla, my advisor thought that I could compensate my excesive solar light intake by spending half a winter in Sweden, in the **Evolutionary Biology Center** in **Uppsala**. Mats Björklund was then and has always been a superb host, allowing me to feel at home in the department of Animal Ecology. Reija Dufva taught to me how to play CSI with birds, and introduced me to Jesus and Moses, the PCR pets of the lab. Despite my continuous singing of swedish radio commercials, Kachenka always had a smile

for me. My office mate, Matt, was always up to any wild idea (we still have to carve a new Diny!) and together we tried very hard to drive Kasia crazy, but she kept on smiling!

Matt and Kasia were probably the first witnesses of the subtle changes of my priorities in life: When I first landed in Arlanda, I was wearing a t-shirt claiming my love for every swedish girl I could find, and my idea of happiness was to be the eternal bachelor. A week later, I moved in with Mirjam, and today, almost three years later (!?!), I'm writing this from her kitchen...Niclas was so proud of her in that crayfish party... Talking about parties, that's something you guys know how to do! I'd like to let everyone know that Sweden was actually quite bright also in winter, thanks to people like Amber (and her laughing!), Richard, Sara B., Simone, Alexei, Paolo, Björn (these last two guys and Mirjam were my R teachers!), Lauren, Cosima, Andreas, Erem, Meghan, Murielle, Hanna, Hanne, Olivia (legendary baywatch spex!), Mårten and Jossan (if you are reading this, stop and let's plan a trip!), Johanna, Marlen, my climbing pals: Henry (put a banana in your ear!), Berni, Lára (I'm flying!) , Silja, Beke (an der ecke), Daniel and Tony. Omar y María (y Marquitos!), Arild (wrong spex!), Alex H, Alex GV (se te echa de menos en Uppsala!), Alex K (star of our volley team) and the rest of the international volley team 2011, Pía, Jessica, Lisa (mis padres te adoran!), Boggie and Natalia (good old original volley team!), Masahito (dancing master!). Yoshi, Leanne and Rado (Lavern survivors!), Simon (thanks for letting me borrow your table while you were in the field!), Julieta (bienvenida!), Phillip, Sophie (which I actually met in Barcelona!) and many others that were and are making Sweden my new home.

I also had the opportunity to work with Anders Møller, in **Orsay** (France). Anders tried to teach me how to write a paper in one day, and he succeeded! However, it only works when I spend two months fooling around with the manuscript before actually beginning to write. I will be eternally grateful for the delicious food I enjoyed during Ander's family dinners. Ismael Galván gave me invaluable help with the spectrophotometer. Franck Courchamp was like a second advisor, let me bore french students to death with my bird stories and made me feel part of his group. And that was very convenient, because it was quite hard to make new friends in Orsay! But two superheroines from Franck's group came to the rescue. Gloria, Cleo and me had heaps of fun saving the world, preparing sushi and enjoying the local cuisine (*ma crêpe*). Laure also showed me around the first days, and then I met Laetitia, Yuya, Celine (Cielín!), Lucille and even some guy from Uranus that could drink liquid nitrogen... We all managed to escape some nights to Paris, and after many years, I met again my old friend Sarah! I did not learn much French, but one thing I do remember: N'importe quoi!!

My last "official" stay was in Auburn, Alabama, and I couldn't possibly have enjoyed it more. Geoffrey Hill and Wendy Hood provided me with their guidance, while still letting me pursue every wild idea. Thanks for your enthusiasm, encouragement and friendship. It was great to have you guys visiting in Barcelona! I was properly introduced to the beautiful bluebirds by Dave, Ryan, Luke and Andrew (thanks for showing me the raptors!), we had lots of fun trying to trick those smart fellows into our nets and traps. The house finches were not so smart, were they, A.J.? (We have yet to shoot "Rise of the Planet Finch"). Emily helped us finding our way in the enclosures (and let me catch the snake!), and, in the lab, Lauren "Ringmaster" organization skills prevented us from going crazy. Geoff and Wendy shared their scientific enthusiasm with these and other members of a great team: Nandini, Christina, Molly, Susan, Chris, Amy... During my stay in Auburn, I had the luck to live with my niiice roomie Laurie, in a great house, with great neighbours, thanks to the not less niiiice Kt. They introduced me to the heart of Alabama in the form of a bunch of friends that took great care of me during my whole stay: Chris, Kasey, Jeana, Ryan (thanks for teaching me flyfishing!), Brandon, Katie, Dana, Flynt (who made us feel like (owning) a million bucks in that amazing lake house!), Jeremy and Megan (you must be paella masters by now!). Thank you all for the kayak trips, the chicken wings (and

that whole chicken I won!), the Dude and the Jesus... Mirjam and me are looking forward to come back there, where the skies are so blue :-).

Por último, quiero dar las gracias a los que más tiempo llevan dándome apoyo incondicional. Mi familia: Mis tíos y primos, mi abuelo, que sabe más estrellas que el guía del Planetario, mi Superabuela, siempre alegre y vital, y mi otra abuelita que aún sigue llamando "solete".

Gracias a mis padres, por su paciencia, ánimos... y por soportar todas las arañas, escorpiones, reptiles, anfibios, quirópteros, luciérnagas, caracoles, grillos, hormigas, escarabajos... (Fernan! Hay una caja que se mueve en el garaje!). Gracias a mi hermanita Lars y a Jho (Compañero-hermano-amigo-cuñao) por poner bonita esta tesis y ser nuestros buddies bajo el mar. And to Mirjam, *sunshine of my bike*, for the incredible amount of work you have done in every stage of this project. For your passionate encouragement and your continuous, priceless support. And for making me believe that, by your side, everything is possible.

PAPERS

List of papers included in this doctoral dissertation and contributions of the co-authors

Mateos-Gonzalez, F., Quesada, J. & Senar, J. C. (2011). Sexy birds are superior at solving a foraging problem. 7(5):668-9. Biology Letters. DOI: 10.1098/rsbl.2011.0163. **Impact factor 2010: 3.651.** Conceived and designed the experiments: FMG, JQ, JCS. Performed the experiments: FMG, JQ. Analyzed the data: FMG, JQ, JCS. Wrote the paper: FMG, JQ, JCS.

Mateos-Gonzalez, F. & Senar, J. C. (2012). Melanin-based trait predicts individual exploratory behaviour in siskins, *Carduelis spinus*. 83(1):229-232. Animal Behaviour. DOI:10.1016/j.anbehav.2011.10.030. **Impact factor 2010: 3.101.** Conceived and designed the experiments: FMG, JCS. Performed the experiments: FMG. Analyzed the data: FMG, JCS. Wrote the paper: FMG, JCS.

Barceló, M., **Mateos-Gonzalez, F.** & Senar, J. C. Conspecific nest defence is reflected in carotenoid and melanin-based traits. (Manuscript) Conceived and designed the experiments: MB, FMG, JCS. Performed the experiments: MB, FMG. Analyzed the data: MB, FMG, JCS. Wrote the paper: MB, FMG, JCS.

Mateos-Gonzalez, F., Senar, J. C. & Björklund, M. True colours? Extra-pair paternity is reflected in both melanin and carotenoid-based colouration in great tits *Parus major*. (Manuscript). Conceived and designed the experiments: MB, FMG, JCS. Performed the experiments: FMG. Analyzed the data: FMG, JCS. Wrote the paper: FMG, MB, JCS.

Senar J. C., **Mateos-Gonzalez, F.**, Uribe, F.& Arroyo Ll. Better the devil you know?: In matters of siskin mate choice, familiarity adds to attractiveness. (In revision in *Behavioral Ecology and Sociobiology*). **Impact factor 2010: 2.565.** Conceived and designed the experiments: JCS, LlA. Performed the experiments: JCS, FMG, LlA, FU. Analyzed the data: JCS, FMG. Wrote the paper: JCS, FMG, FU, LlA.

The papers above will not be part of any other doctoral dissertation.

Barcelona, June 3, 2012.

Dr. Joan Carles Senar Jordà

PAPER 1

Sexy birds are superior at solving a foraging problem

Fernando Mateos-Gonzalez, Javier Quesada and Juan Carlos Senar

Resumen en castellano

Las coloraciones amarillas, rojas o naranjas, basadas en carotenoides, que adornan el plumaje de los machos en muchas especies de aves, funcionan a menudo como señales indicadoras, ofreciendo a las hembras que buscan pareja información acerca de características del macho tales como su condición corporal, su salud o su habilidad para encontrar alimento. Sin embargo, esta general "habilidad para encontrar alimento" no se ha podido definir exactamente hasta ahora. En este estudio pudimos constatar que machos de lúgano (*Carduelis spinus*) más ornamentados muestran una mayor habilidad resolviendo problemas de forrajeo. Estos resultados ponen de manifiesto que la evolución podría haber proporcionado a las hembras una herramienta para evaluar las habilidades cognitivas de sus potenciales parejas.

b i o l o g y
letters

Animal behaviour

Sexy birds are superior at solving a foraging problem

Fernando Mateos-Gonzalez[1,*], Javier Quesada[1,2] and Juan Carlos Senar[1]

[1]*Behavioural and Evolutionary Ecology Research Unit (CSIC), and*
[2]*Catalan Ornithological Institute, Natural History Museum of Barcelona, Pº Picasso s/n, 08003 Barcelona, Spain*
Author for correspondence (fernandomateos@gmail.com).

Yellow, red or orange carotenoid-based colorations in male birds are often a signal to prospecting females about body condition, health status and ability to find food. However, this general 'ability to find food' has never been defined. Here we show that more brightly ornamented individuals may also be more efficient when foraging in novel situations. The results highlight the fact that evolution may have provided females tools to evaluate cognitive abilities of the males.

Keywords: *Carduelis spinus*; problem-solving; foraging ability; coloration

1. INTRODUCTION

The plumage of many bird species is coloured by carotenoids. Since carotenoid pigments must necessarily be acquired from food, it has long been suggested that bright coloration in males signals their ability to procure these compounds [1–3]. It is not yet known, however, what exactly is implied by this ability.

The siskin (*Carduelis spinus*) is a small finch that has a yellow stripe on its wings. The length of this stripe is assessed by females when selecting a mate [4]. The aim of the present study was to investigate whether the size of this patch could be used to discriminate between males that perform differentially a foraging task. As an index of this ability, we examined how siskins performed in accessing food from a partially blocked feeder. The task required birds to remove one or two toothpicks from the feeder in order to reach the pine seeds below. We used the time taken to access the food as a measure of performance.

2. MATERIAL AND METHODS

Male siskins (*C. spinus*) used in the experiments ($n = 29$) were captured in northeast Spain in the autumn and winter of 2007–2008. Birds were separated in groups of four and kept in cages provided with eight feeders spaced along 1 m. This prevented competition for food within the group. To estimate overall patch size, we measured the length of the yellow stripe on the primary wing feather six (P6) (descendent) from the distal edge of primary covert four to the distal end of the yellow colouring, according to Senar & Escobar [5]. This measurement is strongly correlated with the size of the whole yellow patch [5]. Siskins also show a black badge that reflects their dominance within the social group. The size of this black badge is uncorrelated with the yellow wing strip length [6,7]. We also measured the size of this black patch, following Senar *et al.* [6]. Four hours before the trial, birds were isolated and food-deprived to foster motivation. After fasting, a small feeder containing pine seeds was placed on an elevated platform in each test cage. Access to the pine seeds was partially blocked by toothpicks that were criss-crossed from side to side through lateral holes in the feeder. The toothpicks protruded 4 cm on each side. The birds could see the

food, but they were unable to reach it without removing at least one of the sticks. They could do this by moving them sideways, until one end of the stick slid out of the side hole in which it was inserted.

An observer measured the time spent by each bird from the moment they landed on the platform or on the feeder trying to get the food, until the moment when they solved the problem, reaching the pine seeds. If the bird did not go on the platform within 3 min, the trial was considered ended. The maximum time set to solve the problem once birds reached the platform was 5 min. Each bird was observed for a maximum of five trials. If, for instance, a bird got the food source in the third trial, after 62 s, we computed $300 + 300 + 62$ s as the total time needed to solve the problem.

We analysed, using generalized linear models (GLM), differences in the length of the yellow wing stripe, measured on primary six ($n = 29$), between slow and fast problem solvers, including age (yearling versus adult) as a covariate. We defined slow solvers as birds that either did not solve the problem or needed more than one trial to solve the problem (greater than 300 s). Fast solvers were defined as birds that solved the problem within the first trial (less than 300 s). Time to solution was then related to the length of the yellow wing stripe, age, size of the black badge and size of the bird (as measured from tarsus length), using a Cox proportional hazards regression model ($n = 21$). All analyses were implemented in the R statistical computing environment, v. 2.12.2 [8]. Cox regressions were applied using the *survival* package [9].

3. RESULTS

Results showed that fast problem solvers had longer yellow wing stripes than slow problem solvers (GLM: *t*-value = 3.65, $p < 0.01$; figure 1). Birds with longer yellow wing stripes solved the problem faster than those with shorter stripes (table 1 and figure 2). Neither age, nor size of the black bib, nor size of the bird (as measured from tarsus length) had any significant effect on solving time (table 1). Latency time to land on the platform/feeder was not related to the length of the yellow wing stripe (Cox analysis: $z = 0.03$, $p = 0.98$).

4. DISCUSSION

The results of this study showed that the length of the carotenoid-based yellow wing stripe of siskins is related to the ability to solve a foraging problem. This trait did not reflect, though, the latency time to approach the problem, eliminating the possibility of a neophilia effect. The approach we used to test their problem-solving ability may be considered not to measure all aspects of this ability and to be rather artificial. However, we chose this approach because evolution cannot have provided animals with a built-in solution for novel situations of this type, and they have not had a chance to learn what to do through trial and error. This explains why older individuals were not able to take advantage of their experience. Hence, the performance shown while facing this novel task should reflect the ability to solve at least some categories of problems [10]. Challenges of this type are not uncommon in nature, and birds are known to develop singular feeding innovations [11]. A classical example may be the ability of wild tits to manipulate and open milk bottles [12].

Traditionally, the idea that carotenoid-based coloration is related to foraging ability had been tested within the framework linking diet to coloration [3]. Here we show that exploiting carotenoids for colouring ornaments is not only a question of availability and ingestion but also of ability to get them. Moreover, while we do not know yet the breadth of the task
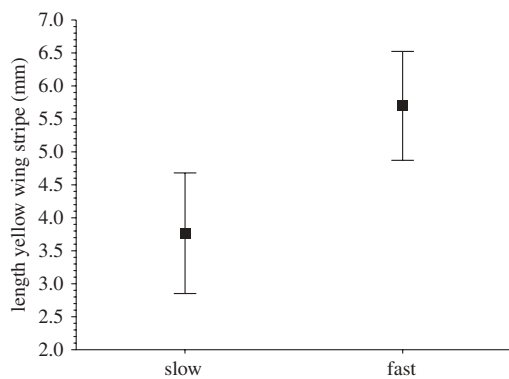
FirstCite
e-publishing

Figure 1. Differences in the length of the yellow wing stripe, measured on primary six ($n = 29$), between slow and fast problem solvers. We defined slow solvers as birds that either did not solve the problem or needed more than one trial to solve the problem (greater than 300 s). Fast solvers are defined as birds that solved the problem within the first trial (less than 300 s). Fast solvers displayed longer yellow wing stripes than slow solvers (s.e. bars shown).
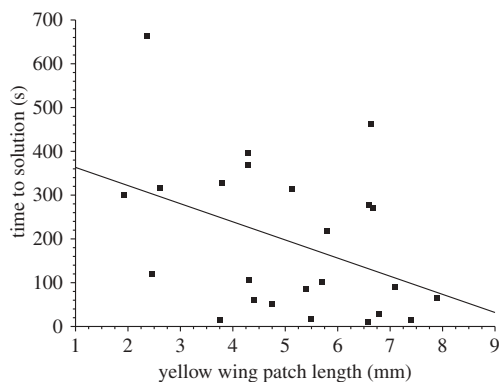

Figure 2. Relationship between the length of the yellow wing stripe ($n = 21$) and the time taken to solve the foraging problem. Birds with longer yellow wing stripes solved the problem faster (table 1). The figure only includes birds that solved the problem.

domain over which our index applies, it also appears that, at least for one dimension of problem-solving, females' choice criteria may be adapted to gauge cognitive performance.

Table 1. Cox proportional hazards analysis relating length of the yellow wing stripe, size of the black bib, age and size of the bird (tarsus length) with the time taken to solve a foraging problem ($n = 21$). (Interactions were not included since they were not significant.)

variable	coef	exp(coef)	s.e.(coef)	z	p-value
yellow wing stripe	0.38	1.47	0.18	2.13	0.03
black bib	-0.94	0.39	0.60	-1.57	0.12
tarsus	-0.37	0.69	0.56	-0.66	0.51
age	-0.02	0.98	0.02	-1.10	0.27

1 Endler, J. A. 1980 Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76–91. (doi:10.2307/2408316).

2 Hill, G. E. 1990 Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Anim. Behav.* **40**, 563–572. (doi:10.1016/S0003-3472(05)80537-8)

3 Hill, G. E. 2006 Bird coloration. In *Mechanisms and measurements*, vol. 1 (eds G. E. Hill & K. J. McGraw), pp. 507–560. Cambridge, MA: Harvard University Press.

4 Senar, J. C., Domènech, J. & Camerino, M. 2005 Female siskins choose mate by the size of the yellow wing stripe. *Behav. Ecol. Sociobiol.* **57**, 445–469. (doi:10.1007/s00265-004-0873-7)

5 Senar, J. C. & Escobar, D. 2002 Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. *Avian Sci.* **2**, 19–24.

6 Senar, J. C., Camerino, M., Copete, J. L. & Metcalfe, N. B. 1993 Variation in black bib of the Eurasian siskin (*Carduelis spinus*) and its role as a reliable badge of dominance. *Auk* **4**, 924–927.

7 Senar, J. C. & Camerino, M. 1998 Status signalling and the ability to recognize dominants: an experiment with siskins (*Carduelis spinus*). *Proc R. Soc. Lond. B* **265**, 1515–1520. (doi:10.1098/rspb.1998.0466)

8 R Development Core Team 2011 *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0. See http://www.R-project.org/.

9 Therneau, T. & Lumley, T. 2011 Survival: survival analysis, including penalised likelihood. R package, version 2.36-5. See http://CRAN.R-project.org/package=survival.

10 Chappell, J. & Kacelnik, A. 2002 Tool selectivity in a non-primate, the New Caledonian crow (*Corvus moneduloides*). *Anim. Cogn.* **5**, 71–78. (doi:10.1007/s10071-002-0130-2)

11 Lefebvre, L. 2000 Feeding innovations and their cultural transmission in bird populations. In *The evolution of cognition* (eds C. Heyes & L. Huber), pp. 311–328. Cambridge, MA: MIT Press.

12 Fisher, J. & Hinde, R. A. 1949 The opening of milk bottles by birds. *Br. Birds* **42**, 347–357.

PAPER 2

Melanin-based trait predicts individual exploratory behaviour in siskins, *Carduelis spinus*

Fernando Mateos-Gonzalez, Javier Quesada and Juan Carlos Senar
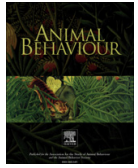
Resumen en castellano

Se conoce como personalidad al conjunto de diferencias individuales en el comportamiento, constantes a lo largo del tiempo y en distintas situaciones. Numerosos trabajos han puesto de manifiesto que estas diferencias comportamentales pueden afectar la eficacia biológica del individuo a través de un gran número de importantes variables, tales como la supervivencia, el éxito reproductor o la dominancia. El importante efecto que la personalidad tiene sobre esas variables puede llegar a determinar asimismo la selección o rechazo de un compañero social o sexual. La existencia de esta presión de selección dependiente de la personalidad sugiere la posibilidad de la aparición de señales que informen de diferentes rasgos comportamentales. Sin embargo, esta probable señalización de la personalidad apenas ha sido considerada. En este trabajo, sugerimos que las aves pueden señalizar rasgos de su personalidad a través del color del plumaje, y probamos esta hipótesis en lúganos (*Carduelis spinus*), relacionando diferentes caracteres del plumaje con la tendencia exploratoria del individuo. Para medir esta tendencia exploratoria, colocamos un objeto novedoso al alcance de cada ave, midiendo el tiempo que cada individuo empleaba en acercarse a dicho objeto nuevo. Descubrimos que lúganos con baberos negros de mayor tamaño mostraban latencias de aproximación más cortas. Este descubrimiento apoya la idea de que ornamentos del plumaje pueden proporcionar información acerca de diferentes rasgos de la personalidad del individuo.

Melanin-based trait predicts individual exploratory behaviour in siskins, *Carduelis spinus*

Fernando Mateos-Gonzalez*, Juan Carlos Senar

Behavioural & Evolutionary Ecology Research Unit (CSIC), Natural History Museum of Barcelona

Individual behavioural differences, consistent over time and/or across situations, have been included under the term of personality. These behavioural differences have been shown to affect a variety of major ecological traits, all of which have a strong effect on the fitness of the individual. However, the personality of an individual could also have a strong influence on the decisions of other individuals, in situations such as selecting or avoiding a social companion or a mate. Under this scenario, indicators of different personalities would have evolved, but the possible signalling of personality has received little consideration to date. We hypothesized that birds can signal their personality through feather coloration and tested this hypothesis in siskins relating different colour patches to their exploration score. To measure exploration, we presented the siskins with a novel object and calculated how long they took to approach it. We found that siskins with larger black bibs showed shorter approach latencies. This finding supports the idea that plumage coloration traits can provide information about personality traits of the bearer.

© 2011 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Personality describes the phenomenon by which individual behavioural differences are consistent over time and/or across situations (Réale et al. 2007). These differences affect a variety of ecological traits, such as dominance, natal dispersal, offspring recruitment, territory quality and reproductive performance, survival and physiological responses to social stress (Réale et al. 2007), all of which have a strong effect on fitness (Smith & Blumstein 2007). Personality variability can be maintained and selected by means of natural selection (Dingemanse & Réale 2005) and/or sexual selection (Schuett et al. 2010). To be sexually selected, personality, like other traits, should be signalled (Andersson 1994). Furthermore, in a social context (Tanaka 1996), these signals could play an important role, for example, when selecting or avoiding a social companion (Senar & Camerino 1998). To be able to assess the personality of a possible social mate could be very relevant. In fact, some researchers have found that an individual's behavioural type can affect its performance in a social-foraging context (Schuett & Dall 2009) and have described how that individual will use social information (Marchetti & Drent 2000; Kurvers et al. 2010a).

Although possible signalling of personality could be of particular interest, few studies on this subject have been performed. A recent study by Garamszegi et al. (2008), however, found that features of birdsong could correlate with estimates of personality, suggesting that females could determine and select personality characters likely to enhance breeding success through a singing-related trait.

Feather coloration could be another channel in birds to inform about personality. Ducrest et al. (2008) proposed a link between melanin-based coloration and personality and this link has been already found in salmonid fishes (Kittilsen et al. 2009) and eastern Hermann's tortoises, *Eurotestudo boettgeri* (Mafli et al. 2011).

In birds, dark areas of coloration based on melanin have been found to function as dominance signals, giving information about their bearer's social status and behaviour towards predators and competitors (Senar 2006). In great tits, *Parus major*, for instance, the size of the melanin-based black stripe on the breast has been related to nest defence (Quesada & Senar 2007) and, independently, nest defence has been related to personality (Hollander et al. 2008). Furthermore, some studies have found dominance and personality to be related (Verbeek et al. 1999; Dingemanse et al. 2004; Fox et al. 2009).

Therefore, we could reasonably predict a link between these melanin-based traits and their bearer's personality. Siskins, *Carduelis spinus*, are highly sociable birds, often forming flocks even during the breeding season (Diesselhorst & Popp 1963; Nethersole-Thompson & Watson 1974). These birds have a black bib, based on

* Correspondence: F. Mateos-González, Behavioural & Evolutionary Ecology Research Unit (CSIC), Natural History Museum of Barcelona, P° Picasso s/n, 08003 Barcelona, Spain.
E-mail address: fernandomateos@gmail.com (F. Mateos-Gonzalez).

melanin, whose size reflects their dominance within the social group (Senar et al. 1993; Senar & Camerino 1998).

It is well established that dominant individuals exploit subordinates, taking advantage of their position to get a larger share of the resources (Barnard & Sibly 1981; Pulliam & Caraco 1984). Dominant siskins go further in these demands, even requiring that their subordinates regurgitate food for them (allofeeding; Senar 1984).

Dominant siskins are also more aggressive, beginning more fights than subordinate ones (Senar 1985), and aggressiveness has been repeatedly related to boldness (Sih et al. 2004). According to the 'life history productivity' hypothesis (Biro & Stamps 2008), which proposes that some individuals achieve higher productivity by displaying a particular behaviour, these bigger-badged, dominant, aggressive, and probably bolder individuals should show a proactive 'coping style' (Koolhaas et al. 1999; Sih et al. 2004) and be faster explorers. Indeed, aggressive (Dingemanse & de Goede 2004; Bell & Sih 2007; but see Jones & Godin 2010) and dominant (David et al. 2011) animals tend to be more exploratory. Therefore, we would expect that siskins with larger bibs would also show higher levels of exploration. We tested this hypothesis by measuring the exploration scores of a group of siskins, following Verbeek et al. (1994), and relating those scores to their black bib sizes.

METHODS

Male siskins used in the experiments ($N = 28$) were captured and ringed in northeast Spain in the autumn and winter of 2007–2008. Bib area was estimated following Senar et al. (1993), along with age, tarsus size, weight, and the length of the yellow wing stripe. The latter is a sexual ornament females use when choosing a mate.

Experiments started approximately 2.5 months after capture. Each day of the experiment, six birds were randomly housed in individual cages (1×0.5 m and 0.5 m high) and food deprived for 4 h, prior to the test, to ensure they were motivated to feed. At the beginning of every trial, a novel red box, containing pine seeds, was placed on a platform (10 cm high, 10 cm wide, 25 cm long) inside the cage with the bird. The individual had to reach at least the platform to see the food inside the box. An observer measured the time spent by each bird from the moment the box was placed on the platform until the moment when the bird touched either the platform or the box. We allowed the bird 3 min to go onto the platform (a slightly more conservative latency time than in Verbeek et al. 1994). If the bird did not go onto the platform within those 3 min, the trial was considered ended and the next bird was tested, so ensuring that no more than 20 min passed between the first and last bird tested. We performed a maximum of five trials per bird. Latency to approach the box was measured as the total time, pooled across trials, until the bird touched the platform or the box for the first time.

The response included censored observations (birds that did not approach the novel object). Applying standard statistical methods to censored data, or not taking them into account, can lead to biased estimates. A semiparametric survival analysis approach deals with this problem neatly (Budaev 1997). Hence we applied a stratified Cox proportional hazards regression model, a specialized nonparametric regression survival analysis, to relate latency to the length of the yellow wing stripe, age, size of the black bib, size of the bird (as measured from tarsus length) and weight ($N = 28$). All analyses were implemented in the R statistical computing environment, version 2.12.2 (R Development Core Team 2011). Cox regressions were applied using the survival package (Therneau & Lumley 2011).

RESULTS

Individuals with larger black bibs showed a shorter latency to approach the novel object (Fig. 1). Age, size, weight and the length of their yellow wing stripe did not have any influence on this latency (Table 1).

DISCUSSION

In line with our prediction, siskins with larger black bibs were also more likely to approach a novel object, denoting a higher level of exploration. Siskins with larger black bibs had previously been found to be dominant within the group (Senar et al. 1993; Senar & Camerino 1998) and also more aggressive (Senar 1985),
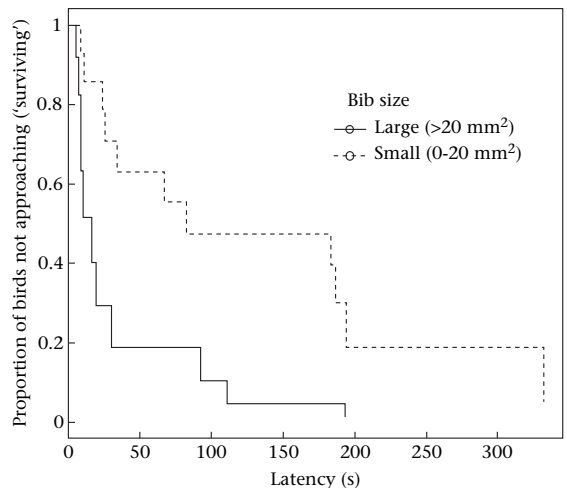

Figure 1. Survivorship plot function for tendency to approach a novel object. It shows the proportion of siskins that did not approach the object ('surviving') up to the respective time interval. Birds ($N = 28$) are represented according to the size of their black bib, which shows a high bimodality in the population (Ripoll et al. 2004).

Table 1
Cox proportional hazards analysis relating size of the black bib, length of the yellow wing stripe, age, weight and size of the bird (tarsus length) to the approach latency time to a novel object ($N = 28$)

	Coefficient	Exp (coefficient)	SE (coefficient)	z	$\Pr(>|z|)$
Black bib size	0.05	1.05	0.02	2.11	0.03
Yellow wing stripe	0.01	1.01	0.17	0.04	0.97
Age	−0.02	0.98	0.54	−0.04	0.97
Tarsus length	−0.93	0.39	0.60	−1.55	0.12
Weight	−0.27	0.76	0.42	−0.65	0.52

Interactions were not included since they were not significant.

a behavioural trait repeatedly related to boldness (Sih et al. 2004). Hence, we suggest that siskins with larger bibs are showing a proactive 'coping style' (Koolhaas et al. 1999; Sih et al. 2004).

Even though our test was performed only once, we are confident of the repeatability of the exploration trait. There is strong support for consistent individual differences (Bell et al. 2009) and, as argued in Fox et al. (2009), individual measures of exploratory behaviour in similar species are typically repeatable for long periods (e.g. Verbeek et al. 1994; Dingemanse et al. 2002; Drent et al. 2003; Quinn & Cresswell 2005).

Melanin-based coloration is linked to several physiological, morphological and behavioural traits (Roulin 2004) and it has been proposed that these links originate in the pleiotropic effects of the genes regulating the synthesis of melanin (Ducrest et al. 2008). Accordingly, it has been found that, for example, darker eumelanic barn owl, *Tyto alba*, nestlings cope better with stress, releasing less corticosterone after a stressful event (Almasi et al. 2010). Dominant siskins, bearing larger eumelanic bibs, also seem to handle stress better than subordinates, according to their metabolic rate (Senar et al. 2000). This greater ability to cope with stress could be the reason behind their faster response to a novel object and should thus reflect a superior ability to handle, for example, the stressful factors of a different environment (Almasi et al. 2008; Roulin et al. 2008).

Foraging tactics, as in the producer–scrounger game (Barnard & Sibly 1981), have been found to be affected by personality (Kurvers et al. 2010b) and the signalling of different personalities could help to shape these foraging tactics.

In siskins, the ability to solve foraging problems is signalled by a different trait: the length of a yellow wing stripe (Senar & Escobar 2002; Mateos-Gonzalez et al. 2011). This ability to solve problems is not related to the size of the black bib. However, a bird's way of approaching a foraging problem (and its chances of finding food) might be very different depending on its personality. It is known that females choose their mates by the size of this yellow wing stripe (Senar et al. 2005) and that foraging siskins tend to avoid conspecifics showing larger black bibs, probably to avoid possible aggressive interactions (Senar & Camerino 1998). It is very probable, then, that both signals have a shared influence when siskins are choosing a mate or a social companion. The length of the yellow wing stripe gives valuable information about the foraging ability of the individual, but surely siskins can also assess their companions' personality through their melanin traits and, thus, they might be able to refine their selection. Hence, most probably, siskins are not only trying to keep away from aggressive birds with larger black bibs but also actively selecting smaller black-badged, slow-explorer individuals as social companions. The latter, apart from being less aggressive, are lesser risk-takers (van Oers et al. 2004) and their personality helps them to identify and avoid potentially dangerous aposematic prey (Exnerová et al. 2010). Despite their greater wariness, slow birds are active foragers that explore their environment more thoroughly than faster birds (Verbeek et al. 1994). They could therefore be a potential source of high-quality social

information and this additional worth could be assessed a priori using this personality signal, which would help to modulate the response to the length of the yellow wing stripe.

Could there then be some selective advantage for dominant, fast-explorer birds? These dominant, fast explorers with large bibs could have a potential survival advantage, owing to their preferential access to resources. However, in this species, dominant individuals mainly fight among themselves, in like-versus-like aggression (Senar et al. 1989, 1997), leaving lower-ranking individuals relatively free from aggression. Also, female siskins do not choose mates based on this trait (Senar et al. 2005) and, accordingly, theoretical models suggest that the relationships among survival, reproductive and aggression rates of dominants and subordinates probably maintain the evolutionary stability of the system, nicely reflected by the binomial frequency distribution of bib sizes in nature (Ripoll et al. 2004). Additionally, empirical studies suggest that, in these 'feudal' species, in which dominants fight only among themselves, both classes even manage to achieve similar survival rates (Senar & Domènech 2011), so it is likely that these are two alternative, equally valid strategies.

In conclusion, our results support the idea that black, melanin-based traits that act as badges of social status within the group probably also reflect inner qualities about the personality of the bearer.

Acknowledgments

References

Almasi, B., Roulin, A., Jenni-Eiermann, S. & Jenni, L. 2008. Parental investment and its sensitivity to corticosterone is linked to melanin-based coloration in barn owls. *Hormones and Behavior*, **54**, 217–223.

Almasi, B., Jenni, L., Jenni-Eiermann, S. & Roulin, A. 2010. Regulation of stress response is heritable and functionally linked to melanin-based coloration. *Journal of Evolutionary Biology*, **23**, 987–996.

Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.

Barnard, C. J. & Sibly, R. M. 1981. Producers and scroungers: a general model and its application to captive flocks of house sparrows. *Animal Behaviour*, **29**, 543–550.

Bell, A. M. & Sih, A. 2007. Exposure to predation generates personality in three-pined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters*, **10**, 828–834, doi:10.1111/j.1461-0248.2007.01081.x.

Bell, A. M., Hankison, S. J. & Laskowski, K. L. 2009. The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, **77**, 771–783.

Biro, P. A. & Stamps, J. A. 2008. Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, **23**, 361–368.

Budaev, S. V. 1997. The statistical analysis of behavioural latency measures. *ISCP Newsletter*, **14**, 1–4.

David, M., Auclair, Y. & Cézilly, F. 2011. Personality predicts social dominance in female zebra finches, *Taeniopygia guttata*, in a feeding context. *Animal Behaviour*, **81**, 219–224.

Diesselhorst, G. & Popp, K. 1963. Zeisigbruten bei Memmingen. *Vogelwelt*, **84**, 184–190.

Dingemanse, N. J. & de Goede, P. 2004. The relation between dominance and exploratory behavior is context-dependent in wild great tits. *Behavioral Ecology*, **15**, 1023–1030.

Dingemanse, N. J. & Réale, D. 2005. Natural selection and animal personality. *Behaviour*, **142**, 1159–1184.

Dingemanse, N. J., Both, C., Drent, P. J., van Oers, K. & van Noordwijk, A. J. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour*, **64**, 929–937.

Dingemanse, N. J., Both, C., Drent, P. J. & Tinbergen, J. M. 2004. Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society B*, **271**, 1541–1547.

Ducrest, A.-L., Keller, L. & Roulin, A. 2008. Pleiotropy in the melanocortin system, colouration and behavioural syndromes. *Trends in Ecology & Evolution*, **23**, 502–510.

Drent, P. J., van Oers, K. & van Noordwijk, A. J. 2003. Realized heritability of personalities in the great tit (*Parus major*). *Proceedings of the Royal Society B*, **270**, 45–51.

Exnerová, A., Hotová Svádová, K., Fucíková, E., Drent, P. & Stys, P. 2010. Personality matters: individual variation in reactions of naive bird predators to aposematic prey. *Proceedings of the Royal Society B*, **277**, 723–728.

Fox, R. A., Ladage, L. D., Roth, T. C. & Pravosudov, V. V. 2009. Behavioural profile predicts dominance status in mountain chickadees *Poecile gambeli*. *Animal Behaviour*, **77**, 1441–1448, doi:10.1016/j.anbehav.2009.02.022.

Garamszegi, L., Eens, M. & Török, J. 2008. Birds reveal their personality when singing. *PLoS One*, **3** (7), 2647.

Hollander, F., Van Overveld, T., Tokka, I. & Matthysen, E. 2008. Personality and nest defence in the great tit (*Parus major*). *Ethology*, **114**, 405–412, doi:10.1111/j.1439-0310.2008.01488.x.

Jones, K. A. & Godin, J. G. J. 2010. Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proceedings of the Royal Society B*, **277**, 625–632, doi:10.1098/rspb.2009.1607.

Kittilsen, S., Schjolden, J., Beitnes-Johansen, I., Shaw, J. C., Pottinger, T. G., Sørensen, C., Braastad, B. O., Bakken, M. & Øverli, Ø 2009. Melanin-based skin spots reflect stress responsiveness in salmonid fish. *Hormones and Behavior*, **56**, 292–298.

Koolhaas, J. M., Korte, S. M., de Boer, S. F., van der Vegt, B. J., van Reenen, C. G., Hopster, H., de Jong, I. C., Ruis, W. & Blokhuis, H. J. 1999. Coping styles: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, **23**, 925–935.

Kurvers, R., van Oers, K., Nolet, B. A., Jonker, R. M., van Wieren, S. E., Prins, H. H. T. & Ydenberg, R. C. 2010a. Personality predicts the use of social information. *Ecology Letters*, **13**, 829–837.

Kurvers, R., Prins, H. H. T., van Wieren, S. E., van Oers, K., Nolet, B. A. & Ydenberg, R. C. 2010b. The effect of personality on social foraging: shy barnacle geese scrounge more. *Proceedings of the Royal Society B*, **277**, 601–608.

Mafli, A., Wakamatsu, K. & Roulin, A. 2011. Melanin-based coloration predicts aggressiveness and boldness in captive eastern Hermann's tortoises. *Animal Behaviour*, **81**, 859–863.

Marchetti, C. & Drent, P. J. 2000. Individual differences in the use of social information in foraging by captive great tits. *Animal Behaviour*, **60**, 131–140.

Mateos-Gonzalez, F., Quesada, J. & Senar, J. C. 2011. Sexy birds are superior at solving a foraging problem. *Biology Letters*, Published online 30 March 2011. doi:10.1098/rsbl.2011.0163.

Nethersole-Thompson, A. & Watson, A. 1974. *The Cairngorms*. London: Collins.

van Oers, K., Drent, P. J., de Goede, P. & van Noordwijk, A. J. 2004. Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. *Proceedings of the Royal Society B*, **271**, 65–73.

Pulliam, H. R. & Caraco, T. 1984. Living in groups: is there an optimal group size? In: *Behavioural Ecology: an Evolutionary Approach* 2nd edn. (Ed. by J. R. Krebs & N. B. Davies), pp. 122–147. Oxford: Blackwell Scientific.

Quesada, J. & Senar, J. C. 2007. The role of melanin- and carotenoid-based plumage coloration in nest defence in the great tit. *Ethology*, **113**, 640–647.

Quinn, J. L. & Cresswell, W. 2005. Personality, anti-predation behaviour and behavioural plasticity in the chaffinch *Fringilla coelebs*. *Behaviour*, **142**, 1377–1402.

R Development Core Team 2011. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. http://www.R-project.org/.

Réale, D., Reader, S., Sol, D., McDougall, P. & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews of the Cambridge Philosophical Society*, **82**, 291–318.

Ripoll, J., Saldaña, J. & Senar, J. C. 2004. Evolutionary stable transition rates in a stage-structured model. An application to the analysis of size distributions of badges of social status. *Mathematical Biosciences*, **190**, 145–181.

Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews*, **79**, 815–848.

Roulin, A., Gasparini, J., Bize, P., Ritschard, M. & Richner, H. 2008. Melanin-based colorations signal strategies to cope with poor and rich environments. *Behavioral Ecology and Sociobiology*, **62**, 507–519.

Schuett, W. & Dall, S. R. X. 2009. Sex differences, social context and personality in zebra finches, *Taeniopygia guttata*. *Animal Behaviour*, **77**, 1041–1050.

Schuett, W., Tregenza, T. & Dall, S. 2010. Sexual selection and animal personality. *Biological Reviews of the Cambridge Philosophical Society*, **85**, 217–246.

Senar, J. C. 1984. Allofeeding in Eurasian siskins (*Carduelis spinus*). *Condor*, **86**, 213–214.

Senar, J. C. 1985. Interactional rules in captive siskins (*Carduelis spinus*). *Miscelanea Zoologica*, **9**, 347–360.

Senar, J. C. 2006. Bird colors as intrasexual signals of aggression and dominance. In: *Bird Coloration. 2. Function and Evolution* (Ed. by G. E. Hill & K. J. McGraw), pp. 125–193. Cambridge, Massachusetts: Harvard University Press.

Senar, J. C. & Camerino, M. 1998. Status signalling and the ability to recognize dominants: an experiment with siskins (*Carduelis spinus*). *Proceedings of the Royal Society B*, **265**, 1515–1520.

Senar, J. C. & Domènech, J. 2011. Sex-specific aggression and sex ratio in wintering finch flocks: serins and siskins differ. *Acta Ethologica*, **14**, 7–11, doi:10.1007/s10211-010-0084-3.

Senar, J. C. & Escobar, D. 2002. Carotenoid derived plumage coloration in the siskin *Carduelis spinus*. *Avian Sciences*, **2**, 19–24.

Senar, J. C., Camerino, M. & Metcalfe, N. B. 1989. Agonistic interactions in siskin flocks: why are dominants sometimes subordinate? *Behavioral Ecology and Sociobiology*, **25**, 141–145.

Senar, J. C., Camerino, M., Copete, J. L. & Metcalfe, N. B. 1993. Variation in black bib of the Eurasian siskin (*Carduelis spinus*) and its role as a reliable badge of dominance. *Auk*, **110**, 924–927.

Senar, J. C., Camerino, M. & Metcalfe, N. B. 1997. A comparison of agonistic behaviour in two Cardueline finches: feudal species are more tolerant than despotic ones. *Etología*, **5**, 73–82.

Senar, J. C., Polo, V., Uribe, F. & Camerino, M. 2000. Status signalling, metabolic rate and body mass in the siskin: the cost of being subordinate. *Animal Behaviour*, **59**, 103–110.

Senar, J. C., Domènech, J. & Camerino, M. 2005. Female siskins choose mate by the size of the yellow wing stripe. *Behavioral Ecology and Sociobiology*, **57**, 445–469, doi:10.1007/s00265-004-0873-7.

Sih, A., Bell, A. M., Johnson, J. C. & Ziemba, R. E. 2004. Behavioral syndromes: an integrative overview. *Quarterly Review of Biology*, **79**, 241–277.

Smith, B. R. & Blumstein, D. T. 2007. Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, **19**, 448–455.

Tanaka, Y. 1996. Social selection and the evolution of animal signals. *Evolution*, **50**, 512–523.

Therneau, T. & Lumley, T. 2011. Survival: Survival analysis, including penalised likelihood. R package version 2.36-5. http://CRAN.R-project.org/package=survival.

Verbeek, M. E. M., Drent, P. J. & Wiepkema, P. R. 1994. Consistent individual differences in early exploratory behaviour of male great tits. *Animal Behaviour*, **48**, 1113–1121.

Verbeek, M. E. M., De Goede, P., Drent, P. J. & Wiepkema, P. R. 1999. Individual behavioural characteristics and dominance in aviary groups of great tits. *Behaviour*, **136**, 23–48.

PAPER 3

Conspecifc nest defence is reflected in both melanin and carotenoid-based traits in the great tit *Parus major*

Margarida Barceló, Fernando Mateos-Gonzalez and Juan Carlos Senar

Manuscript

Resumen en castellano

El plumaje del carbonero común (*Parus major*) incluye una banda negra ventral cuya coloración está basada en melaninas. Este ornamento funciona como una señal social, cuyo tamaño refleja la dominancia y agresividad del individuo. Esta señal también ha sido relacionada con el grado de defensa del nido contra predadores: Aves con bandas mayores defienden más intensamente sus nidos contra predadores que individuos con bandas menores. Los carboneros son muy territoriales y también defienden sus nidos de intrusos de su misma especie, protegiendo sus recursos e intentando evitar pérdidas de eficacia biológica en forma de infidelidad. Podría esperarse, entonces, que la misma señal basada en melanina también estuviera relacionada con esta defensa contra conespecíficos. En este estudio, examinamos la respuesta de machos de carbonero a intrusiones experimentales de conespecíficos. Pudimos observar que la respuesta agonística de los machos residentes dependía de la interacción entre la corbata negra, basada en melaninas, y la coloración amarilla, basada en carotenoides, del pecho de estos machos residentes. Aquellos machos cuyo plumaje amarillo mostraba altos niveles de saturación y, al mismo tiempo, presentaban una corbata negra de mayor tamaño, defendieron de forma menos intensa su nido que individuos que mostraban sólo altos niveles de calidad en uno de los dos ornamentos.
Estos resultados sugieren que los machos de mayor calidad podrían estar invirtiendo más en estrategias alternativas con un beneficio potencialmente mayor, tales como la búsqueda de cópulas extra-pareja, en la que probablemente serán más exitosos que otros machos con ornamentos de menor calidad.

Conspecific nest defence is reflected in both melanin and carotenoid-based traits in the great tit *Parus major*

MARGARIDA BARCELÓ, FERNANDO MATEOS-GONZALEZ
& JUAN CARLOS SENAR

Behavioural & Evolutionary Ecology Research Unit (CSIC), Natural History Museum of Barcelona.

Great tits show a melanin-based black tie that works as a social signal, reflecting dominance and aggression levels. This trait has also been related to nest defence against predators: birds with larger black ties defend more intensely their nests in front of a predator than individuals with smaller ties. great tits are also highly territorial against conspecifics, defending their resources and trying to avoid fitness loss in form of cuckoldry. It would be expected, then, that the melanin-based trait would give information about its bearer's level of nest defence also against conspecifics. In this study we tested the response of male great tits against an experimental conspecific intrusion. Our results showed that the agonistic response of resident males against conspecific intrusions was driven by the interaction between the black tie size and the carotenoid yellow colouration of the chest. Males with large black ties, whose yellow chest plumage showed also higher levels of chroma – a trait often related to individual quality – displayed a less intense conspecific nest defence than males with either only a large black tie or high levels of chroma. These results suggest that males of highest quality might be investing more in alternative strategies with a higher revenue in fitness, such as the search of extra-pair copulations, in which they can be more successful than males with duller traits.

Plumage colouration is known to be a reliable indication of individual quality that often plays a major role in sexual selection and social competition (Rohwer & Rohwer 1978; McGraw 2006a, b). This colouration results, among others, from the microstructure of the feathers, from pigment deposition such as melanins and carotenoids (Fox 1976; Brush 1978), or from different combinations of these (Hill & McGraw 2006).

Animals acquire carotenoids only by ingestion (Goodwin 1950; Brush 1978) and, as a result, ornaments based on these pigments are often related to foraging abilities (Hill & Montgomerie 1994; Senar & Escobar 2002; Hill & McGraw 2006; Mateos-Gonzalez et al. 2011). However, melanins are synthesized by the individual itself and melanin-based traits are instead often involved in intrasexual competition, as signals of social status (Rohwer 1975;

Correspondence: M. Barceló, Behavioural & Evolutionary Ecology Research Unit (CSIC), Natural History Museum of Barcelona, Pº Picasso s/n, 08003 Barcelona, Spain. Tel.(0034)676785107, Fax:(0034)971660310, email: margabarcelo5@gmail.com

Senar 1999, 2006).

Great tits (*Parus major*) are small passerine birds that present both kinds of signals: a typical melanin-based ventral black tie, crossing bright yellow, carotenoid-based plumage (Lemel & Wallin 1993; Senar 2006; Quesada & Senar 2007). Since long, it is known that a larger black tie implies more dominance and aggressiveness in this species (Järvi & Bakken 1984; Pöysä 1988). Additionally, males with larger, melanin-based, black ties have been found to defend their nests more intensely against predators (Norris 1990; Quesada & Senar 2007).

Great tits also defend their nest and surroundings against conspecific intrusions (Slagsvold et al. 1994). Usually, these intrusions are performed by males looking for extra-pair copulations (EPC) (Lubjuhn et al. 1999). This behaviour can provide important benefits to the intruder, such as a direct fitness increase, a potential new mate acquisition or, should the intruder be already paired, an insurance in case of social mate infertility. The resident female can gain genetic diversity and quality, and, like the intruder, compensate for a potential infertility prob-

lem of the social partner (Birkhead & Møller 1992). An important reason to avoid conspecific intrusions is that the resident male faces a risk of losing within pair paternity (Westneat & Stewart 2003). Great tits also try to avoid conspecific intrusions to maintain their own territory (Slagsvold et al. 1994), especially their nest hole, a limited resource for which there is high competition (East & Perrins 1988; Gustafsson 1988; Newton 1994; Yom-Tov 2001).

Given that individuals with a larger black tie are known to be more aggressive (Järvi & Bakken 1984; Senar 2006; Quesada & Senar 2007) and defend their nest more strongly against predators (Quesada & Senar 2007), we could predict that males with larger black ties should also show a more intense defensive behaviour against conspecific intruders. We tested this prediction by experimentally simulating interspecific intrusions, measuring the response of local males and relating this response to their plumage colouration.

METHODS

The experiment was carried out at Can Catà field station, during the breeding season of 2011. This station is located in a Mediterranean mixed forest, with deciduous oak (*Quercus cerrioides*) at the bottom of the valleys and aleppo pine (*Pinus halepensis*) forest on the hills, near the city of Barcelona (northeast Iberian Peninsula) (see Figuerola & Senar (2005) for more details). There were 179 nest boxes distributed in the study area (80ha), mainly occupied by an established population of great tits.

At the beginning of the breeding season, mid March, we started visiting the nest boxes every three days to establish nest state, laying day, hatching day and brood size.

Since nest attendance is highest during brood rearing this increases the prospects of the resident male to detect an intrusion. We decided to perform our experiments during this period, when nestlings were between nine and ten days old. Neighbour intrusions are most frequent during the female fertile period (Ford 1983; Møller 1987), but latter intrusions are equally repelled, for example, to keep the highly valuable nest holes (Gustafsson 1988). We could expect that these latter conspecific intrusions would receive similar levels of rejection as earlier ones, given that aggressiveness is a personality trait that shows relative consistency across contexts and situations (Sih et al. 2004; Dingemanse & Réale 2005; Réale et al. 2007). Accordingly, previous work in great tits has shown no significant differences in male aggressive behaviour against conspecifics between egg laying and later stages (Janssens et al. 2003).

The nest defence behaviour of resident males was recorded following van Duyse et al. (2002) and Janssens et al. (2003). We placed a live male great tit decoy in a small cage, on top of every nest box (N=23), to simulate a territorial intrusion. At the start of the test, we played 30 seconds of a great tit song, at a natural volume, meant to attract the attention of the nest owner. The observer was hidden in a car at 10-15 meters away from the nest box. When there was no reaction from the resident male in 5 minutes, the playback was used again during 30 additional seconds.

Once the resident male was spotted, we observed the aggressive behaviour of the resident male during 10 minutes. The aggressive behaviour of the resident male was estimated using four parameters: 1) minimum distance at which the male approached the decoy bird (Distance), 2) number of direct aggressive attacks performed by the resident male to the decoy (Attacks), 3) number of aggressive displays carried out by the resident male to the decoy (Displays), and 4) total time the resident male spent on top of the decoy's cage (TT).

When the chicks were 15 days old, their parents were captured. Parents and nestlings were ringed, and tarsus length measured. Sex and age of birds were determined according to Svensson (1992) and Jenni & Winkler (1994). We measured the size of the black tie area taking digital photos and processing them with Image Tool 2.0 software, following Figuerola & Senar (2000) and Quesada & Senar (2007). We also took yellow plumage colouration values using a Minolta CR200 portable colorimeter, which provides independent values of hue, chroma and lightness following Figuerola et al. (1999).

As we did not know *a priori* which conspecific nest defence behaviours were of importance, and we wished to avoid making an arbitrary choice of variables, we performed a principal component analysis (PCA) with all the defence measures.

We retained the first factor for further analyses (eigenvalue >1), this component could be taken as a composite measure of defensive behaviour (Quesada & Senar 2007; Table 1). Then we performed a multiple regression to analyse the effect on territorial defence of plumage colouration, tarsus length, age of the resident male, number of nestlings and date of the experiment, and the interactions between the different colour parameters. Lightness was not included in the model, as this variable was correlated to chroma. The full model was then stepwise reduced until the final model contained only factors with an associated $p < 0.10$, to produce the best-fit model (Sokal & Rohlf 1995).

All statistical tests were performed using R v. 2.12.2 (R development core team 2011). The graph (Fig. 1) was made using Statistica software v. 8.

Table 1. Principal component analysis (PCA) of territorial nest defence variables. Eigenvalue for PC1=1.62. Cumulative proportion of explained variance = 65%. (TT=total time the resident male spent on top of the decoy's cage).

	PC1 Factor loadings
Distance	0.40
Attacks	-0.51
Displays	-0.48
TT	-0.59

Ethical note

The great tit used as a decoy was captured in Sarrià, a suburban area in the outskirts of Barcelona (Spain), an

area independent from the study field station, not to interfere with the study population.

It was captured in mid March, when the odds of it having a dependent brood were highly unlikely. The bird was trapped with a special funnel trap used as a permanent feeding location when not activated, and transported to our laboratory in a small aluminium and plastic cage (20x20x15), covered with a dark cloth.

In our laboratory the bird was housed, in an aluminium cage (80x40x40 cm) located in a quiet place with natural sunlight and isolated from human external sounds. Inside we placed four wooden perches and a small wooden nest box (20x15x10 cm).

The bird was provided with *ad libitum* fresh water, and food (a mix of: living worms (*Zophobas morio*), pate of insects (Orlux), fruit supplement (Vitackaft fruit cocktail) and peanuts). As food supplements the bird received vitamins, antibiotics and antifungals dissolved in the drinking water (Omni-Vit; Canariz).

During the tests the bird was housed in a small aluminium and plastic cage (20x20x15) with two plastic perches, and provided with water and food. Between the tests the cage was covered with a dark cloth and placed in a quiet place not to stress the bird unnecessarily. We performed a maximum of two-three tests per day. Only on a few occasions did the tested resident male spend more than a minute in contact with the cage (TT), but it never made physical contact with the decoy bird. The decoy bird responded to the displays but never seemed very stressed.

The decoy bird did not lose weight during captivity, and was released healthy in the same place of capture by the end of May.

Animals tested in the wild (adults and nestlings) were captured inside their nest boxes. We took colour and biometrical data and, as quickly as possible, we released them again inside their nest box. Birds were handled, ringed and maintained in captivity under permission of the Catalan Ringing Office (ICO), and following the Catalan standards for the use of animals in research.

Table 2. Multiple backward regression model of territorial nest defence (PC1) on black tie size (mm^2), chroma and hue of the carotenoid-based yellow plumage of the chest, brood size, date of the experiment, age and tarsus length of the resident male, and interactions between the different colour parameters (Full model). $F_{10,12}=2.35$, p=0.08.

Variable	Estimate	SE	t	p
Black tie size	-0.86	0.46	-1.87	0.09
Chroma	-2.23	4.04	-0.55	0.59
Hue	-2.99	2.21	-1.35	0.20
Brood size	-0.09	0.26	-0.32	0.75
Age	-0.76	0.72	-1.06	0.31
Tarsus length	0.29	0.69	0.43	0.68
Date	-0.008	0.10	-0.09	0.93
Chroma*Black tie size	0.004	0.001	3.45	0.005
Hue*Black tie size	0.007	0.005	1.70	0.12
Chroma*Hue	0.008	0.04	0.22	0.83

RESULTS

Our results show that the nest defence response was modulated by an interaction between black tie size and yellow breast plumage colouration (Tables 2, 3 and Fig. 1). Males with large black ties, whose yellow chest plumage also showed higher levels of chroma, displayed a less intense conspecific nest defence than males with either only a large black tie or high levels of chroma (Tables 2, 3 and Fig. 1). We found no effects of hue, age, tarsus length, number of nestlings or date of the experiment.

Table 3. Final model of multiple backward regression of territorial nest defence (PC1) on black tie size (mm^2), chroma of the carotenoid-based yellow plumage of the chest and interaction between the two. $F_{3,19}=7.49$, p=0.002.

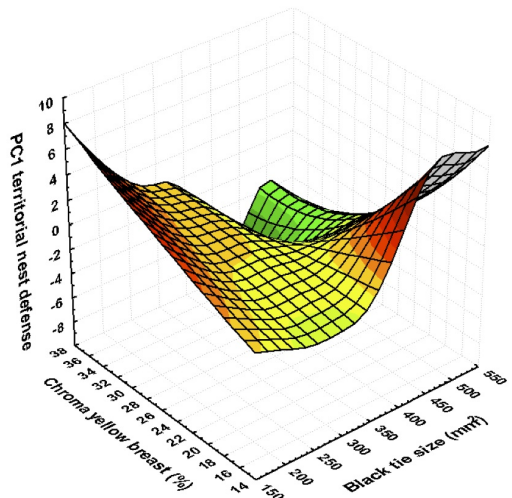| Variable	Estimate	SE	t	p
Black tie size	-0.09	0.02	-4.05	<0.001
Chroma	-1.23	0.36	-3.43	0.002
Chroma*Black tie size	0.004	0.001	3.77	0.001

Figure 1. Relationship between nest defence (PC1), chroma of the yellow chest and black tie size (mm^2).

DISCUSSION

Our initial prediction was that the black tie size would determine the level of nest defence of male great tits against a conspecific intrusion. However, the black tie was not the only trait involved in this response. The agonistic behaviour of resident males against conspecific intrusions was driven by the interaction between the black tie size and the carotenoid yellow colouration of the chest. Males with large black ties, whose yellow chest plumage also showed higher levels of chroma, displayed a less intense conspecific nest defence than males with either only a large black tie or high levels of chroma.

When Quesada & Senar (2007) tested the nest defence of great tits against a predator intrusion, they did not find any such effect of the yellow breast colouration on the response against a predator. This fact could be due to the different nature of the intrusions. In the face of a predator attacking his nest, a male great tit only has two simple alternatives: to risk his life trying to save his offspring or to let them die. In this situation, probably only his own aggressiveness (reflected in his melanin-based trait; Järvi & Bakken 1984; Senar 2006; Quesada & Senar 2007), and his potential loss of investment, in terms of brood size, will have an influence on his decision (Quesada & Senar 2007). Individual qualities such as foraging ability, immunocompetence or parasitic resistance, which are signalled by yellow carotenoid-based colouration (McGraw & Hill 2000a,b; Senar et al. 2003; McGraw & Ardia 2007), are irrelevant in this extreme situation. From the predator's point of view, this conspicuous yellow colouration will not very likely make a difference either, given the dissimilarity in colour vision between these species and common avian predators (Håstad et al. 2005). As a consequence, a lack of association between carotenoid-based colouration and nest defence in the face of predators is expected (Quesada & Senar 2007).

However, a conspecific intrusion may not be such a drastic event, assuming a lower risk for the resident male and his offspring. In this situation, his investment in terms of brood size would not be so critical. Accordingly, in opposition to Quesada & Senar (2007), our current experiment showed that the number of nestlings did not have an effect on the male's response.

Nevertheless, a conspecific intrusion could imply a potential decrease in fitness in the form of loss of within pair paternity (Westneat & Stewart 2003), or the loss of the nest hole, an important limiting factor for this species (East & Perrins 1988; Gustafsson 1988; Newton 1994; Yom-Tov 2001). Therefore, a choice must be made, but now, depending on his individual quality, a male could choose between investing more in conspecific nest defence and mate guarding or instead trying to improve his fitness by pursuing EPC (Kokko & Morrell 2005). Following this hypothesis, we could describe three different groups of males, according to their quality and potential strategy:

Birds showing small black tie sizes and low yellow chroma values are likely to be in poor condition (Galvan & Alonso-Alvarez 2008; Senar et al. 2008). They are signalling a low individual quality, which probably makes them less sexually attractive, and therefore, less able to successfully achieve EPCs (Kempenaers et al. 1992). Their low condition would probably also hamper their nest defence/mate guarding. Furthermore, females paired with these low quality males would be expected to actively seek EPCs (Kempenaers et al. 1992; Kempenaers 1995). Probably, despite any mate guarding effort, these males may be doomed to lose paternity (Kempenaers 1995). This uncertainty of paternity could make them even more prone to reduce their investment in nest defence (Lubjuhn et al. 1993).

Males in average condition, enjoying an intermediate attractiveness, either based on yellow chroma or black tie size, might be able to achieve more EPCs than lower quality ones. However, they could still find it more beneficial to ensure their within-pair paternity by enhancing their mate guarding strategies. In fact, not so sexually attractive individuals have been proposed to follow a compensatory strategy by increasing their aggressiveness (McGraw et al. 2007), mate guarding (Johnsen & Lifjeld 1995), and by investing more in parental care (Sanz 2001). In turn, when females detect higher parental effort in their mates, they are less likely to seek extra-pair mating opportunities during second broods (O'Brien & Dawson 2010). This positive feedback could again reassure the male's investment in the nest (Lubjuhn et al. 1993). In fact, in the great tit, both yellow chroma (Broggi & Senar 2009) and black tie size (Norris 1990) have been related to parental investment.

Finally, males that display both high values of yellow chroma and large tie sizes are signalling a higher quality, hence they should be expected to be more sexually attractive. For these males, it could simply be more profitable not to invest in conspecific nest defence, given that agonistic encounters with other males can still be a risky activity (Senar 2006). High quality males could instead greatly improve their fitness by pursuing EPCs (Kokko & Morrell 2005). Being attractive, these males would be more likely to get EPCs (Birkhead & Møller 1992; Bitton et al. 2007; Balenger et al. 2009). It is even possible that, despite their lack of commitment, their females would still keep faithful to them (Kempenaers et al. 1992; Kempenaers et al. 1997). Even if the lack of nest defence leads to the possibility of being cuckolded, the net balance in fitness could still be positive for a sexually attractive male great tit. In conclusion, while male great tits with a larger melanin-based black tie area defend their nests more intensely against a predator attack (Quesada & Senar 2007), in the case of a conspecific intrusion, the response would be modulated both by that trait and the carotenoid-based yellow colouration of the breast. Most probably, resident males are following different strategies, according to their sexual attractiveness and their chances to achieve successful EPCs. Further studies, directly testing links between colouration and extra-pair paternity are clearly imperative, and could help to clarify to which extent these different strategies are successful.

REFERENCES

Balenger, S., Johnson, L. & Masters, B. 2009. Sex-

ual selection in a socially monogamous bird: male color predicts paternity success in the mountain bluebird, *Sialia currucoides*. Behavioral Ecology and Sociobiology, 63, 403–411.

Birkhead, T. R. & Møller, A. P. 1992. Sperm Competition in Birds. London: Academic Press.

Bitton, P. P., O'Brien, E. L. & Dawson, R. D. 2007. Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor*. Animal Behaviour, 74, 1777–1784.

Broggi, J. & Senar, J. C. 2009. Brighter Great tit parents build bigger nests. Ibis, 151, 588–591.

Brush, A. H. 1978. Avian pigmentation. In: Chemical Zoology, Vol. X, Aves (Ed. by A. H. Brush), pp. 141–161. New York: Academic Press.

Dingemanse, N. & Réale, D. 2005. Natural selection and animal personality. Behaviour, 142, 1159–1184.

East, M. L. & Perrins, C. M. 1988. The effect of nest-boxes on breeding populations of birds in broadleaves temperate woodlands. Ibis, 130, 393–401.

Figuerola, J. & Senar, J. C. 2000. Measurement of plumage badges: an evaluation of methods used in the Great Tit *Parus major*. Ibis, 142, 482–484.

Figuerola, J. & Senar, J. C. 2005. Seasonal changes in carotenoid- and melanin-based plumage coloration in the Great Tit *Parus major*. Ibis, 147, 797–802.

Figuerola, J., Pascual, J. & Senar, J. C. 1999. The use of a colorimeter in field studies of Blue Tit *Parus caeruleus* coloration. Ardea, 87, 269–275.

Fox, D. L. 1976. Animal Biochromes and Structural Colours. Berkeley, CA: University of California Press.

Ford, N. L. 1983. Variation in mate fidelity in monogamous birds. Current Ornithology, 1, 329–356.

Galvan, I. & Alonso-Alvarez, C. 2008. An intracellular antioxidant determines the expression of a melanin-based signal in a bird. Plos ONE, 310, e3335.

Goodwin, T. W. 1950. Carotenoids and reproduction. Biological Reviews, 25, 391–413.

Gustafsson, L. 1988. Inter- and intraspecific competition for nest holes in a population of the Collared Flycatcher *Ficedula albicolis*. Ibis, 130, 11–16.

Håstad, O., Victorsson, J. & Ödeen, A. 2005. Differences in color vision make passerines less conspicuous in the eyes of their predators. Proceedings of the National Academy of Sciences of the United States of America, 102, 6391–6394.

Hill, G. E. & McGraw, K. J. 2006. Bird Coloration. Volume 2. Function and Evolution. Cambridge: Harvard University Press.

Hill, G. E. & Montgomerie, R. 1994. Plumage colour signals nutritional condition in the house finch. Proceedings of the Royal Society of London series B, 258, 47–52.

Janssens, E., Dauwe, T., van Duyse, E., Beernaert, J., Pinxten, R. & Eens, M. 2003. Effects of heavy metal exposure on aggressive behavior in a small territorial songbird. Archives of Environmental Contamination and Toxicology, 45, 121–127.

Järvi, T. & Bakken, M. 1984. The function of the variation in the breast stripe of the Great tit (*Parus major*). Animal Behaviour, 32, 590–596.

Jenni, L. & Winkler, R. 1994. Moult and Ageing of European Passerines. London: Academic Press.

Johnsen, A. & Lifjeld, J. T. 1995. Unattractive males guard their mates more closely: An experiment with bluethroats (Aves, Turdidae: *Luscinid-s- suecica*). Ethology, 101, 200–212.

Kempenaers, B. 1995. Polygyny in the blue tit: Intra- and inter-sexual conflicts. Animal Behaviour, 49, 1047–1064.

Kempenaers, B., Verheyen, G. R. & Dhondt, A. A. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behavioral Ecology, 8, 481–492.

Kempenaers, B., Verheyen, G. R., van den Broeck, M., Burke, T., van Broeckhoven, C. & Dhondt, A. A. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. Nature, 357, 494–496.

Kokko, H. & Morrell, L. J. 2005. Mate guarding, male attractiveness, and paternity under social monogamy. Behavioral Ecology, 16, 724–731.

Lemel, J. & Wallin, K. 1993. Status signalling, motivational condition and dominance: an experimental study in the great tit, *Parus major* L. Animal Behaviour, 45, 549–558.

Lubjuhn, T., Curio, E., Muth, S. C., Brün, J. & Epplen, J. T. 1993. Influence of extra-pair paternity on parental care in great tits (*Parus major*). In: DNA Fingerprinting: State of the Science (Ed. by S. D. J. Pena, R. Chakraborty, J. T. Epplen & A. J. Jeffreys), pp. 379-385. Basel/Switzerland: Birkhäuser Verlag.

Lubjuhn, T., Strohbach, S., Brun, J., Gerken, T. & Epplen, J. T. 1999. Extra-pair paternity in great tits (*Parus major*) a long term study. Behaviour, 136, 1157–1172.

Mateos-Gonzalez, F., Quesada, J. & Senar, J. C. 2011. Sexy birds are superior at solving a foraging problem. Biology Letters, 7, 668–669.

McGraw, K. J. 2006a. Mechanics of Carotenoid-based Coloration. In: Bird Coloration, Volume 1, Mechanisms and Measurements (Ed. by G. E. Hill & K. J. McGraw), pp. 177–242. Cambrigde, MA: Harvard University Press.

McGraw, K. J. 2006b. Mechanics of Melanin-based Coloration. In: Bird Coloration, Volume 1, Mechanisms and Measurements (Ed. by G. E. Hill & K. J. McGraw) Cambrigde, MA: Harvard University Press.

McGraw, K. J. & Hill, G. E. 2000a. Carotenoid-based ornamentation and status signaling in the house finch. Behavioral Ecology, 11, 520–527.

McGraw, K. J. & Hill, G. E. 2000b. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proceedings of the Royal Society of London series B, 267, 1525–1531.

McGraw, K. J. & Ardia, D. R. 2007. Do carotenoids buffer testosterone-induced immunosuppression? An experimental test in a colourful songbird. Biology Letters, 3, 375–378.

McGraw, K. J., Medina-Jerez, W. & Adams, H.

2007. Carotenoid-based plumage coloration and aggression during molt in male house finches. Behaviour, 144, 165–178.

Møller, A. P. 1987. Intruders and defenders on avian breeding territories: The effect of sperm competition. Oikos, 48, 47–54.

Newton, I. 1994. The role of nest sites in limiting the numbers of hole-nesting birds: a review. Biological conservation, 70, 265–276.

Norris, K. J. 1990. Female choice and the quality of parental care in the great tit *Parus major*. Behavioral Ecology and Sociobiology, 27, 275–281.

O'Brien, E. L. & Dawson, R. D. 2010. Plumage color and food availability affect male reproductive success in a socially monogamous bird. Behavioral Ecology, 22, 66–72.

Pöysä, H. 1988. Feeding consequences of the dominance status in Great Tit *Parus major* groups. Ornis Fennica, 65, 69–75.

Quesada, J. & Senar, J. C. 2007. The role of melanin- and carotenoid-based plumage coloration in nest defence in the Great Tit. Ethology, 113, 640–647.

R Development Core Team. 2011. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: http://www.R-project.org.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews, 82, 291–318.

Rohwer, S. A. 1975. The social significance of avian winter plumage variability. Evolution, 29, 593–610.

Rohwer, S. A. & Rohwer, F. C. 1978. Status signalling in Harris sparrows: experimental deceptions achieved. Animal Behaviour, 26, 1012–1022.

Sanz, J. J. 2001. Experimentally reduced male attractiveness increases parental care in the pied flycatcher *Ficedula hypoleuca*. Behavioral Ecology, 12, 171–176.

Senar, J. C. 1999. Plumage coloration as a signal of social status. In: Proc. 22 Int. Ornithol. Congr., Durban (Ed. by N. Adams & R. Slotow), pp. 1669–1686.

Johannesburg: BirdLife South Africa.

Senar, J. C. 2006. Color Displays as Intrasexual Signals of Aggression and Dominance. In: Bird Coloration, Volume 2: Function and Evolution (Ed. by G. E. Hill & K. J. McGraw) Cambridge, MA.: Harvard University Press.

Senar, J. C. & Escobar, D. 2002. Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. Avian Science, 2, 19–24.

Senar, J. C., Figuerola, J. & Domènech, J. 2003. Plumage coloration and nutritional condition in the Great tit *Parus major*: the roles of carotenoids and melanins differ. Naturwissenschaften, 90, 234–237.

Senar, J. C., Negro, J. J., Quesada, J., Ruiz, I. & Garrido, J. 2008. Two pieces of information in a single trait?: the yellow breast of the Great Tit reflects both pigment acquisition and body condition. Behaviour, 145, 1195–1210.

Sih, A., Bell, A. & Johnson, J. C. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution. 19, 372–378.

Slagsvold, T., Dale, S. & Saetre, G. P. 1994. Dawn singing in the great tit (*Parus major*): Mate attraction, mate guarding, or territorial defence? Behaviour, 131, 115–118.

Sokal, R. R. & Rohlf, F. J. 1995. Biometry. 3rd edn. New York: W. H. Freeman and Company.

Svensson, L. 1992. Identification Guide to European Passerines. 4 edn. Stockholm: L. Svensson.

van Duyse, E., Pinxten, R. & Eens, M. 2002. Effects of testosterone on song, aggression, and nestling feeding behavior in male great tits, *Parus major*. Hormones and Behavior, 41, 178–186.

Westneat, D. F. & Stewart, I. R. K. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. Annual Review of Ecology, Evolution, and Systematics, 34, 365–396.

Yom-Tov, Y. 2001. An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. Ibis, 143, 133–143.

PAPER 4

True colours? Within pair paternity loss is reflected in both melanin and carotenoid-based colouration in great tits *Parus major*

Fernando Mateos-Gonzalez, Juan Carlos Senar and Mats Björklund

Manuscript

Resumen en castellano

Aunque la mayoría de las aves son socialmente monógamas, es muy común hallar infidelidad en muchas especies. El atractivo del macho está considerado como un elemento clave en el comportamiento extra-pareja y, consecuentemente, varios estudios han explorado el efecto que señales basadas en melaninas o carotenoides podrían tener en las probabilidades de sufrir infidelidad en diferentes especies. Sin embargo, pocos de estos trabajos han tenido en cuenta ambos tipos de pigmentos al mismo tiempo en una misma especie. El Carbonero común (*Parus major*) tiene dos ornamentos basados en esos pigmentos, que señalizan diferentes características del individuo. Uno de ellos, el tamaño de la corbata negra basada en melaninas, ha sido relacionado, con diferentes resultados, con el comportamiento extra-pareja de la hembra. En carboneros, las coloraciones basadas en carotenoides funcionan como una señal de calidad pero hasta ahora este ornamento no se ha tenido en cuenta al estudiar los mecanismos del comportamiento extra-pareja en el Carbonero común. En este trabajo, estudiamos el efecto conjunto que las señales basadas en carotenoides y melaninas del carbonero macho podrían tener en el riesgo de sufrir infidelidad. Nuestra predicción fue que la calidad de estos ornamentos se correlacionaría con tasas de infidelidad más bajas. Sorprendentemente, pudimos constatar que los machos de mayor calidad, aquellos que mostraban al mismo tiempo corbatas melánicas mayores y coloraciones amarillas más brillantes, sufrieron tanta infidelidad de parte de sus parejas que aquellos que mostraban ornamentos de la peor calidad, mientras que aquellos que mostraban buena calidad en un solo tipo de ornamento fueron capaces de mantener íntegra su certeza de paternidad. Estos resultados sugieren que, en nuestra población, los machos de mayor calidad pueden estar siguiendo una estrategia distinta, maximizando su paternidad extra-pareja en detrimento de la propia.

True colours? Within pair paternity loss is reflected in both melanin and carotenoid-based colouration in great tits *Parus major*

FERNANDO MATEOS-GONZALEZ[1], JUAN CARLOS SENAR[1] & MATS BJÖRKLUND[2]

[1]Behavioural & Evolutionary Ecology Research Unit (CSIC), Natural History Museum of Barcelona, Pº Picasso s/n, 08003 Barcelona, Spain. [2]Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden.

Even though most birds are socially monogamous, it is highly common to find extra-pair offspring in most species. Male attractiveness is considered to play an important role in extra-pair paternity behaviour. Consequently, a number of studies has explored the effect that melanin or carotenoid-based quality signals might have in the risks of suffering or avoiding cuckoldry in different species. However, up to now, no studies have jointly tested both kind of signals in a single species. In the great tit *Parus major*, two traits based on these pigments are present, signalling different qualities of the individual. One of them, the size of the melanin-based black stripe, has been related, with different results, to extra-pair female choice. In great tits, carotenoid-based colourations are a well known quality signal but, to our knowledge, up to now no studies have taken this trait into account when addressing the mechanisms of extra-pair behaviour. In this study, we tested the joint effect of both carotenoid and melanin-based traits, on the risk of suffering cuckoldry in the great tit. We predicted that the quality of these traits would correlate with lower cuckoldry rates. Contrary to predictions, higher quality males, those showing both larger black stripes and brighter yellow colourations, were as heavily cuckolded as the lowest quality ones, while those showing high values of only one trait were able to keep higher rates of within pair paternity certainty. We propose that, in our population, male great tits might be following a different strategy, depending on their quality.

Key words: *Parus major*, plumage colouration, carotenoids, melanin, extra-pair paternity.

Even though most birds are socially monogamous, it is highly common to find extra-pair offspring in many species (Birkhead & Møller 1992; Birkhead & Møller 1995). Extra-pair sexual behaviour can have important effects on fitness and, consequently, research efforts have been aimed to describe causes and consequences of this behaviour for all parties concerned (Griffith et al. 2002; Westneat & Stewart 2003). Hence, while the motivation for females to engage in extra-pair copulations is not so clear yet, reasons for males to obtain extra pair paternity

Correspondence: Fernando Mateos-Gonzalez; Behavioural & Evolutionary Ecology Research Unit (CSIC), Natural History Museum of Barcelona, Pº Picasso s/n, 08003 Barcelona, Spain. Email: fernandomateos@gmail.com

and keep their within pair paternity intact, are rather obvious (Dixon et al. 1994; Griffith et al. 2002; Mays et al. 2008). Male attractiveness is considered to play an important role in extra-pair paternity (Griffith et al. 2002) and a number of studies have tested the effect of different male quality traits on the risks of suffering cuckoldry, such as song quality (Forstmeier et al. 2002), age (Lubjuhn et al. 2007), condition (Møller et al. 2003), body size (Yezerinac & Weatherhead 1997) or plumage traits (Canal et al. 2011).

Plumage colouration can be, arguably, the most conspicuous quality trait in many birds and, as such, has also received a fair amount of consideration in extra-pair behaviour studies, mainly focusing on the two most common pigments: melanins and carotenoids. However, few stud-

ies have taken both kinds of signals into account, when addressing the mechanisms of extra-pair behaviour in a single species (but see van Dongen & Mulder 2009).

Great tits *Parus major* are a socially monogamous species in which extra-pair paternity occurs on a regular basis (Lubjuhn et al. 1999). These birds show both kinds of signals on their ventral side: a melanin-based black breast stripe, crossing bright carotenoid-based yellow plumage, and these two traits have been found to function as signals of different qualities of the individual. The black stripe has been related to dominance, aggressiveness or nest defence against predator intrusions (Järvi & Bakken 1984; Norris 1990; Quesada & Senar 2007) and has been included in several studies testing possible correlates of male traits to extra-pair paternity behaviour (Krokene et al. 1998; Strohbach et al. 1998; Kawano et al. 2009). Of these authors, only Kawano et al. (2009) found that males with larger black ties suffered less cuckoldry, supporting the "good genes" hypothesis (Kempenaers et al. 1992; Kempenaers et al. 1997). However, neither Krokene et al. (1998) nor Strohbach et al. (1998) found such an effect of the melanin-based trait. Kawano et al. (2009) suggested that one reason for these different results could be the possible variation in adaptive female choice among generations or populations (Colegrave et al. 2002).

Another non-exclusive possibility is that the importance of another variable might have been underestimated. The yellow ventral colouration of the great tit is also a quality trait that has been found to signal different characteristics of the individual, such as foraging ability, immunocompetence performance or parasitic resistance (Horak et al. 2001; Senar et al. 2003; Hegyi et al. 2007; Senar et al. 2008; Eeva et al. 2009). This carotenoid-based trait has, to our knowledge, never been considered when addressing the mechanisms of extra-pair behaviour of great tits. However, a carotenoid supplementation experiment performed by Helfenstein et al. (2008) strongly suggests that males showing brighter yellow plumage would be less prone to suffer paternity loss.

In this study, we tested the joint effect of both carotenoid and melanin-based traits of the Great tit, on the risk of suffering cuckoldry, predicting that individuals of the highest quality, signalled both by a brighter carotenoid-based yellow plumage and a larger melanin-based black tie, would suffer less within-pair cuckoldry than those showing lesser quality traits.

METHODS

Field site and methods

Data from four different breeding seasons were obtained in Can Catà, a field station located in a Mediterranean mixed forest of deciduous oak (*Quercus cerrioides*) and aleppo pine (*Pinus halepensis*), near the city of Barcelona (northeast Iberian Peninsula; see Figuerola & Senar (2005) for more details). There were 180 nest boxes distributed in the study area (80ha), mainly occupied by an established population of great tits. Each breeding season, we monitored the occupied nest boxes to determine hatching date and brood size. When the chicks were 14 days old, parents and nestlings were captured in the nest box and individually ringed with aluminium numbered rings. Sex and age of adults were determined according to Svensson (1992) and Jenni & Winkler (1994). Blood samples were obtained from the brachial vein of each individual. A total of 260 chicks and 84 adults were sampled. Seven of these adults were sampled in at least two breeding seasons.

Colour measurements

To measure the size of the black tie area we took digital photos and processed them with Image Tool 2.0 software, (see Figuerola & Senar (2000) and Quesada & Senar (2007) for more details). We also took yellow plumage colouration values using a Minolta CR200 portable colorimeter, which provides independent values of hue, chroma and lightness (Figuerola et al. 1999).

Paternity analysis

Paternity was analysed using PCR amplification of 8 polymorphic DNA microsatellite loci: Pma179, Pma48m, Pma49m, Pma69u, Pma303 (Kawano 2003) Pca8, Pca7 and Pca9 (Dawson et al. 2000). We followed the polymerase chain reaction protocol described in Dawson et al. (2000) and Kawano (2003).

Data from field observations and paternity analysis results were compared, and nestlings were considered extra-pair if they mismatched their alleged father at two or more loci.

Statistical analysis

We calculated the proportion of extra-pair paternity for each male whose true and extra-pair offspring was known with certainty (n=18). The proportion was calculated as the ratio between extra-pair offspring and true offspring for each nest. So, for example, if a given father had 2 extra-pair chicks in his nest, out of a brood of 5, the proportion of cuckoldry suffered by this father would be 0.4.

We then related this predictor to age of the social father, lightness, hue, chroma of the yellow colouration of his breast, size of his black tie and the possible interactions among these colour variables.

We developed a best-fit statistical model by reducing the full model until the final one only contained factors with an associated $p < 0.10$ (Sokal & Rohlf 1995). Data were analysed with a non-parametric general linear model using ranked data (Conover & Iman 1980, 1982). All analyses were realized with Statistica 8.0; Stat soft. Inc., Tulsa, OK, USA.

RESULTS

Male great tits with larger black ties and higher levels of chroma on their yellow plumage suffered higher rates of cuckoldry than those showing high levels of only one of those colour traits. Birds showing lower levels in both traits also suffered comparatively more cuckoldry (Figure 1, Table 1). Age did not have a significant effect on the chances of suffering cuckoldry.
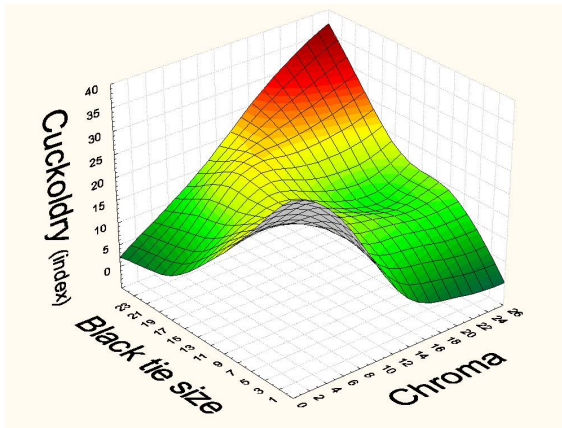
Figure 1. Proportion of extra-pair/within pair paternity in relation to size of the black tie and chroma of the yellow breast colouration of male great tits.

Table 1. Ranked GLM relating size of the black tie, yellow chroma, and their interaction with the proportion of extra-pair/within pair paternity of male great tits (Final model).

	F	β	p
Black tie size	5.88	-1.10	0.02
Yellow chroma	5.66	-1.22	0.03
Black tie size * Yellow chroma	8.32	1.21	0.01

DISCUSSION

Contrary to predictions, birds displaying both high values of yellow chroma and large tie sizes were as heavily cuckolded as those showing low quality traits, while birds with either only a large black tie or high values of yellow chroma were able to maintain a lower rate of extra-pair paternity within their nests.

We did not find any effect of age on the risks of being cuckolded in our population, in accordance with some papers (Krokene et al. 1998; Johannessen et al. 2005) but not with others (Lubjuhn et al. 2007).

The chroma of the yellow breast plumage of the great tit is an indicator of body condition that can work as a quality signal (Senar et al. 2008). To show high quality colour traits did not prevent their bearers from being cuckolded, but led them to suffer even higher cuckoldry rates than less ornamented individuals.

A possible interpretation of these results could be that, in our population, males might be following different strategies according to their individual quality. In great tits, males perform costly paternity assurance behaviours, like mate guarding (Björklund & Westman 1986; Björklund et al. 1992) and song at dawn (Mace 1987). In many species, males showing higher quality traits usually enjoy bigger chances of getting extra pair copulations (Birkhead & Møller 1992; Bitton et al. 2007; Balenger et al. 2009), even regardless of the quality of the male they are cuckolding (Forstmeier 2007). With this promising prospect, attractive male great tits could find it more profitable to invest more in pursuing extra-pair copulations, instead of employing their efforts in costly mate guarding behaviours, even if that increases the chances of compromising their within pair paternity (Kokko & Morrell 2005). In fact, in great tits, a perceived loss of within pair paternity leads in turn to an even lower mate guarding effort (Hansen et al. 2009). This could explain the results found in our population, where, indeed, males of higher quality – those with larger black ties and a brighter yellow colouration – invest less in nest defence against conspecific intrusions than males with high levels of only one of those traits (Barcelò et al. unpublished).

This same strategy has been suggested for some species in which older males suffer more cuckoldry than younger ones (Westneat 1987; Sherman & Morton 1988), and it is also known to be followed, for example, by black-throated blue warblers *Dendroica caerulescens* (Chuang-Dobbs et al. 2001) in which males with more extra-pair opportunities guard less and suffer more cuckoldry.

The alternative tactic would be the one followed by males showing only high levels of either the melanin or the carotenoid-based trait. These individuals, of a suboptimal attractiveness, either based on a high yellow chroma or a large black tie size, seem to suffer less cuckoldry in our population. For these males it would probably be more beneficial to ensure their within-pair paternity by enhancing their mate guarding strategies, given that they would not be so successful when trying to achieve extra-pair copulations. In fact, it has been suggested that not so sexually attractive individuals seem to follow an alternative strategy by increasing their aggressiveness (McGraw et al. 2007), mate guarding (Johnsen & Lifjeld 1995), and parental care (Sanz 2001). This higher parental effort could be rewarded in faithfulness by females (O'Brien & Dawson 2010), which would again, in turn, reinforce the male's investment in the nest (Lubjuhn et al. 1993; Lubjuhn 1995).

Lastly, the males with small tie sizes and low chroma values, signalling a very low quality, could not expect to successfully achieve many extra-pair copulations. Very likely, females paired with these low quality males would be actively seeking for EPCs (Kempenaers et al. 1992; Kempenaers 1995) but, even if these males decide to follow a mate guarding strategy, their low condition (i.e. low yellow chroma; Senar et al. 2008) and low aggressiveness (Senar 2006; Galvan & Alonso-Alvarez 2008) would result in a poor nest defence/mate guarding performance, which would probably lead to the observed paternity loss of these males in our population (Kempenaers 1995). These males are in fact very poorly active in defending their territories (Barceló et al. unpublished).

In conclusion, we found that males of the highest quality, as signalled by carotenoid and melanin-based colour traits, were as heavily cuckolded as those with lowest quality traits, while males of intermediate quality were more successful at keeping their within pair paternity. These results suggest that males might be following different reproductive strategies according to their individual quality. Unfortunately, our data did not allow us to identify the genetic father of the extra-pair offspring. This information would confirm whether male great tits with both melanin

and carotenoid-based high quality traits are indeed successfully achieving more extra-pair copulations, a prediction that arises from our results and should be tested in future works.

Finally, our study highlights the potential importance of the carotenoid-based colouration in sexual selection in the great tit and emphasizes the need of taking into account multiple colour traits in extra-pair paternity studies.

REFERENCES

Balenger, S., Johnson, L. & Masters, B. 2009. Sexual selection in a socially monogamous bird: male color predicts paternity success in the mountain bluebird, *Sialia currucoides*. Behavioral Ecology and Sociobiology, 63, 403–411.

Birkhead, T. R. & Møller, A. P. 1992. Sperm Competition in Birds. London: Academic Press.

Birkhead, T. R. & Møller, A.P. 1995. Extra-pair copulation and extra-pair paternity in birds. Animal Behaviour, 49, 843–848.

Bitton, P. P., O'Brien, E. L. & Dawson, R. D. 2007. Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor*. Animal Behaviour, 74, 1777–1784.

Björklund, M., Møller, A. P., Sundberg J. & Westman, B. 1992. Female great tits, *Parus major*, avoid extra-pair copulation attempts. Animal Behaviour, 43, 691–693.

Björklund, M. & Westman, B. 1986. Mate-guarding in the Great tit: Tactics of a territorial forest-living species. Ornis Scandinavica, 17, 2, 99–105.

Canal, D., Potti, J. & Dávila, J. A. 2011. Male phenotype predicts extra pair paternity in pied flycatchers. Behaviour, 148, 691–712.

Chuang-Dobbs, H. C., Webster, M. S. & Holmes, R. T. 2001. Paternity and parental care in the black-throated blue warbler, *Dendroica caerulescens*. Animal Behaviour, 62, 83–92.

Colegrave, N., Kotiaho, J. S. & Tomkins, J. L. 2002. Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. Evolutionary Ecology Research, 4, 911–917.

Conover, W. J. & Iman, R. L. 1980. The rank transformation as a method of discrimination with some examples. Communications in Statistics Part A-Theory and Methods, 9, 465–487.

Conover, W. J. & Iman, R. L. 1982. Analysis of covariance using the rank transformation. Biometrics, 38, 715–724.

Dawson, D. A., Hanotte, O., Greig, C., Stewart, I. R. K. & Burke, T. 2000. Polymorphic microsatellites in the blue tit *Parus caeruleus* and their cross-species utility in 20 songbird families. Molecular Ecology, 9, 1941–1944.

Dixon, A., Ross, D., O'Malley, L. C. & Burke, T. 1994. Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. Nature, 371, 698–700.

Eeva, T., Sillanpaa, S. & Salminen, J. P. 2009. The effects of diet quality and quantity on plumage colour and growth of great tit *Parus major* nestlings: a food manipulation experiment along a pollution gradient. Journal of Avian Biology, 40, 491–499.

Figuerola, J., Pascual, J. & Senar, J. C. 1999. The use of a colorimeter in field studies of Blue Tit *Parus caeruleus* coloration. Ardea, 87, 269–275.

Figuerola, J. & Senar, J. C. 2000. Measurement of plumage badges: an evaluation of methods used in the Great Tit *Parus major*. Ibis, 142, 482–484.

Figuerola, J. & Senar, J. C. 2005. Seasonal changes in carotenoid- and melanin-based plumage coloration in the Great Tit *Parus major*. Ibis, 147, 797–802.

Forstmeier, W. 2007. Do individual females differ intrinsically in their propensity to engage in extra-pair copulations? Plos ONE, 2, e952.

Forstmeier, W., Kempenaers, B., Meyer, A. & Leisler, B. 2002. A novel song parameter correlates with extra-pair paternity and reflects male longevity. Proceedings of the Royal Society of London. Series B: Biological Sciences, 269, 1479–1485.

Galvan, I. & Alonso-Alvarez, C. 2008. An intracellular antioxidant determines the expression of a melanin-based signal in a bird. Plos ONE, 310, e3335.

Griffith, S. C., Owens, I. P. F. & Thuman, K. A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology, 11, 2195–2212.

Hansen, B. T., Johannessen, L. E. & Slagsvold, T. 2009. Interspecific cross-fostering affects mate guarding behaviour in great tits (*Parus major*). Behaviour, 146, 1349–61.

Hegyi, G., Szigeti, B., Török, J. & Eens, M. 2007. Melanin, carotenoid and structural plumage ornaments: information content and role in great tits *Parus major*. Journal of Avian Biology, 38, 698–708.

Helfenstein, F., Losdat, S., Saladin, V. & Richner, H. 2008. Females of carotenoid-supplemented males are more faithful and produce higher quality offspring. Behavioral Ecology, 19, 1165–1172.

Horak, P., Ots, I., Vellau, H., Spottiswoode, C. & Møller, A. P. 2001. Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. Oecologia, 126, 166–173.

Järvi, T. & Bakken, M. 1984. The function of the variation in the breast stripe of the Great tit (*Parus major*). Animal Behaviour, 32, 590–596.

Jenni, L. & Winkler, R. 1994. Moult and Ageing of European Passerines. London: Academic Press.

Johannessen, L. E., Slagsvold, T., Hansen, B. T. &

Lifjeld, J. T. 2005. Manipulation of male quality in wild tits: effects on paternity loss. Behavioral Ecology, 16, 747–754.

Johnsen, A. & Lifjeld, J. 1995. Unattractive males guard their mates more closely: an experiment with bluethroats (Aves, Turdidae: *Luscinia s. svecica*). Ethology, 101, 200–212.

Kawano, K. 2003. Isolation of polymorphic microsatellite markers in the great tit (*Parus major minor*). Molecular Ecology Notes, 3, 314–316.

Kawano, K. M., Yamaguchi, N., Kasuya, E. & Yahara, T. 2009. Extra-pair mate choice in the female great tit *Parus major*: good males or compatible males. Journal of Ethology, 27, 349–359.

Kempenaers, B. 1995. Polygyny in the blue tit: Intra- and inter-sexual conflicts. Animal Behaviour, 49, 1047–1064.

Kempenaers, B., Verheyen, G. R. & Dhondt, A. A. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behavioral Ecology, 8, 481–492.

Kempenaers, B., Verheyen, G. R., van den Broeck, M., Burke, T., van Broeckhoven, C. & Dhondt, A. A. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. Nature, 357, 494–496.

Kokko, H. & Morrell, L. J. 2005. Mate guarding, male attractiveness, and paternity under social monogamy. Behavioral Ecology, 16, 724–731.

Krokene, C., Rigstad, K., Dale, M. & Lifjeld, J. T. 1998. The function of extrapair paternity in blue tits and great tits: good genes or fertility insurance? Behavioral Ecology, 9, 649–656.

Lubjuhn, T. 1995. Reproductive strategies and parental effort in birds: great tits (*Parus major*) – a case study. Verhandlungen der Deutschen Zoologischen Gesellschaft, 88, 15–21.

Lubjuhn, T., Curio, E., Muth, S. C., Brün, J. & Epplen, J. T. 1993. Influence of extra-pair paternity on parental care in great tits (*Parus major*). In: DNA Fingerprinting: State of the Science (Ed. by S. D. J. Pena, R. Chakraborty, J. T. Epplen & A. J. Jeffreys), 379–385. Basel/Switzerland: Birkhäuser Verlag.

Lubjuhn, T., Gerken, T., Brun, J. & Schmoll, T. 2007. Yearling male great tits, *Parus major*, suffer more strongly from cuckoldry than older males. Zoology, 110, 387–397.

Lubjuhn, T., Strohbach, S., Brun, J., Gerken, T. & Epplen, J. T. 1999. Extra-pair paternity in great tits (*Parus major*) a long term study. Behaviour, 136, 1157–1172.

Mace, R. 1987. The dawn chorus in the great tit *Parus major* is directly related to female fertility. Nature, 330, 745–746.

Mays, H., Albrecht, T., Liu, M. & Hill, G. 2008. Female choice for genetic complementarity in birds: a review. Genetica, 134, 147–158.

McGraw, K., Medina-Jerez, W. & Adams, H. 2007. Carotenoid-based plumage coloration and aggression during molt in male house finches. Behaviour, 144, 165–178.

Møller, A. P., Brohede, J., Cuervo, J. J., de Lope, F. & Primmer, C. R. 2003. Extrapair paternity in relation to sexual ornamentation, arrival date and condition in a migratory bird. Behavioural Ecology, 14, 707–712.

Norris, K. J. 1990. Female choice and the quality of parental care in the great tit *Parus major*. Behavioral Ecology and Sociobiology, 27, 275–281.

O'Brien, E. & Dawson, R. 2010. Plumage color and food availability affect male reproductive success in a socially monogamous bird. Behavioral Ecology, 22, 66–72.

Quesada, J. & Senar, J. C. 2007. The role of melanin- and carotenoid-based plumage coloration in nest defence in the Great Tit. Ethology, 113, 640–647.

Sanz, J. J. 2001. Experimentally reduced male attractiveness increases parental care in the pied flycatcher *Ficedula hypoleuca*. Behavioral Ecology, 12, 171–176.

Senar, J. C. 2006. Color Displays as Intrasexual Signals of Aggression and Dominance. In: Bird Coloration, Volume 2: Function and Evolution (Ed. by G. E. Hill & K. J. McGraw) Cambridge, MA.: Harvard University Press.

Senar, J. C., Figuerola, J. & Domènech, J. 2003. Plumage coloration and nutritional condition in the Great tit *Parus major*: the roles of carotenoids and melanins differ. Naturwissenschaften, 90, 234–237.

Senar, J. C., Negro, J. J., Quesada, J., Ruiz, I. & Garrido, J. 2008. Two pieces of information in a single trait?: the yellow breast of the Great Tit reflects both pigment acquisition and body condition. Behaviour, 145, 1195–1210.

Sherman, P. & Morton, M. 1988. Extra-pair fertilizations in mountain white-crowned sparrows. Behavioral Ecology and Sociobiology, 22, 413–420.

Sokal, R. R. & Rohlf, F. J. 1995. Biometry. 3th edn. New York: W. H. Freeman and Company.

Strohbach, S., Curio, E., Bathen, A., Epplen, J. T. & Lubjuhn, T. 1998. Extrapair paternity in the great tit (*Parus major*): a test of the "good genes" hypothesis. Behavioral Ecology, 9, 388–396.

Svensson, L. 1992. Identification Guide to European Passerines. 4 edn. Stockholm: L. Svensson.

van Dongen, W. F. D. & Mulder, R. A. 2009. Multiple ornamentation, female breeding synchrony, and extra-pair mating success of golden whistlers (*Pachycephala pectoralis*). Journal of Ornithology, 150, 607–620.

Westneat, D. 1987. Extra-pair fertilizations in a predominantly monogamous bird: genetic evidence. Animal Behaviour, 35, 877–886.

Westneat, D. F. & Stewart, I. R. K. 2003. Extrapair paternity in birds: causes, correlates, and conflict. Annual Review of Ecology, Evolution, and Systematics, 34, 365–396.

Yezerinac, S. M. & Weatherhead, P. J. 1997. Extrapair mating, male plumage coloration and sexual selection in yellow warblers (*Dendoica petechia*). Proceedings of the Royal Society of London series B, 264, 527–532.

PAPER 5

Better the devil you know? In matters of siskin mate choice, familiarity adds to attractiveness

Juan Carlos Senar, Fernando Mateos-Gonzalez, Francesc Uribe and Lluïsa Arroyo

Resumen en castellano

Existe una considerable controversia en ecología evolutiva acerca de si la familiaridad social produce atracción o rechazo cuando una hembra selecciona pareja. El estudio de la familiaridad es importante, dado que el potencial de adaptación local de una especie puede verse seriamente afectado por la variación de selección sexual de individuos familiares. Hasta la fecha, sin embargo, la mayoría de estudios que relacionan ornamentación y familiaridad ha sido llevada a cabo casi exclusivamente en especies que evitan la familiaridad. En este estudio, mediante experimentos de selección sexual en lúganos (*Carduelis spinus*), analizamos cómo ornamentos y familiaridad social interactúan para influir la selección de pareja. Los resultados mostraron la preferencia de la hembra por machos conocidos, al seleccionar entre machos familiares y desconocidos, si sus ornamentos sexuales eran similares.

Al seleccionar entre un macho desconocido muy ornamentado y un macho familiar menos ornamentado, las hembras no mostraron una preferencia significativa por uno de los dos. Nuestros resultados sugieren que la familiaridad puede ser tan importante como los ornamentos sexuales a la hora de determinar el comportamiento de la hembra en contextos de selección sexual. Dado que el potencial de adaptacion local puede favorecerse por la preferencia de individuos familiares como pareja, la familiaridad social debería ser considerada un importante factor de selección evolutiva.

Better the devil you know?
In matters of siskin mate choice, familiarity adds to attractiveness

J.C. Senar, F. Mateos-Gonzalez, F. Uribe & Ll. Arroyo

Abstract There is currently considerable controversy in evolutionary ecology revolving around whether social familiarity breeds contempt or attraction when a female chooses a mate. The topic of familiarity is significant because by avoiding or preferring familiar individuals as mates, the potential for local adaptation may be reduced or favored.

To date, however, the few studies investigating the interaction between familiarity and ornamentation have only been conducted in species that avoid familiarity. Here, we provide data on mate choice experiments in siskins, analyzing how familiarity and patterns of ornamentation interact to influence mating success. Results showed a female preference for familiar individuals when choosing between familiar and unfamiliar males with similar sized wing patches (i.e. ornaments). When confronting females with the choice between a highly ornamented unfamiliar male and a less ornamented familiar male, half of the females still preferred the social familiar birds as mates.

Our finding suggests that male familiarity may be as important as sexual ornaments in affecting female behavior in mate choice. Given that the potential for local adaptation may be favored by preferring familiar individuals as mates, social familiarity should be regarded as an important evolutionary selection pressure.

Keywords: Familiarity, siskin, *Carduelis spinus,* local adaptation.

Introduction

It has long been assumed that social familiarity should breed contempt when a female chooses a mate (Kelley *et al*. 1999; Mariette *et al*. 2010). Preference for unfamiliar mates is thought to be favored because it reduces inbreeding and increases gene flow (Johnson *et al.* 2010).

J.C. Senar, F. Mateos-Gonzalez, F. Uribe & Ll Arroyo
Behavioural & Evolutionary Ecology Research Unit (CSIC),
Natural History Museum of Barcelona,
Pº Picasso s/n, 08003 Barcelona, Spain.
tlf. +34.932562216; Fax: +34.933104999
e-mail: jcsenar@bcn.cat

However, a detailed review of the literature has shown that familiarity may also lead to attraction (Cheetham *et al.* 2008). The authors reported that, in species with a low inbreeding risk, choosing a familiar individual as mate can be beneficial as it ensures a male that has already demonstrated its quality, health and social status (Cheetham *et al.* 2008).

When familiarity influences female mating preferences, the strength of sexual selection on male ornamentation could be altered (Zajitschek *et al.* 2006). As sexual selection may play a key role in speciation (Panhuis *et al*. 2001; Price 2008; Mank 2009; van Doorn *et al*. 2009), the topic of familiarity is significant. It goes beyond the subject of mating systems, because by avoiding or preferring familiar individuals as mates, the potential for local adaptation and even speciation may be reduced or favored. To date, however, the few studies investigating the interaction between familiarity and ornamentation have only been conducted in species that avoid familiarity (Patricelli *et al*. 2003; Zajitschek *et al*. 2006; Zajitschek & Brooks 2008).

Here we used the siskin (*Carduelis spinus*), a small cardueline finch, to analyze how familiarity and patterns of ornamentation interact to influence mating success. The siskin has a yellow stripe on its wings and the size of this stripe is related to an individual's ability to find food (Senar & Escobar 2002; Mateos-Gonzalez *et al*. 2011). Previous work showed that in mate-choice trials with unfamiliar males, females showed a marked preference for males with a larger wing patches (Senar *et al.* 2005). Here we tested female preference when choosing between familiar and unfamiliar males with similar sized wing patches (i.e. ornaments). We then determined the relative strength of familiarity and ornamentation by confronting females with the choice between a highly ornamented unfamiliar male and a less ornamented familiar male.

Methods

Siskins used in the experiments were captured in NE Spain in the autumn and winter of 2007-2008. On capture we measured the length of the yellow stripe on primary six, from the distal edge of primary covert four to the distal end of the yellow stripe, according to Senar & Escobar (2002). This measure on primary six correlates highly with the size of the whole yellow patch and was thus used to estimate its size (Senar & Escobar 2002). Age was assessed according to Svensson (1992) and

Martin (1994), distinguishing yearlings (birds in their first winter) from adults.

Except during experiments, the birds were kept in indoor cages (100x40x40 cm) with natural light, in flocks of one female and two males. One of the males had a large (L), yellow wing patch, while the other had a small (S) yellow wing patch (average patch for L=7.1 mm; S=3.8 mm; average difference between the two individuals within the "group": 3.3±SD:1.0 mm; paired t-test: t=12.7, p<0.001). To ensure familiarity between the three birds they remained in their same group for at least two months over the winter before starting the mate choice experiments. Previous studies have determined that familiarity in siskins is attained after 20 days of cohabiting in the same cage (Senar *et al.* 1990). Our period of at least 2 months is therefore more than sufficient time for the three birds to become familiar.

Mate-choice trials were conducted in the spring of 2008, following the experimental set up of Hill (1990). We used a mate-choice box that consisted of a central chamber measuring 100x100x100 cm (cage C). The box had two side compartments measuring 20x20x30 cm (cages A and B). A plexiglas window was attached to an exterior cage at the end of each side compartment. During each trial, the two exterior cages each contained one stimulus male bird and the main chamber housed the test female. The test female could view only one stimulus male at a time. To do so, the female had to hop under one of the two doorways (10 cm high) that led to a side compartment (A and B) and jump up on to a perch (for details see (Burley *et al*. 1982; Hill 1990; Senar *et al*. 2005). Females were released into the central chamber between 14:00 and 15:00h on the day before the experiment. Passing the night in the experimental cage allowed them to become accustomed to the cage well in advance of the experiment.

Both the central (C) and the side (A and B) compartments contained infra-red detectors connected to a computer. The computer automatically recorded the time that the female stayed in each compartment. Trials lasted four hours, timed from the moment the computerized system detected that the female had visited both stimulus males. All trials were conducted from 9:00 h, when males were introduced into the side cages. Whether a given male was housed in compartment A or B was chosen at random, thereby eliminating positioning effects. No observers were present in the experimental room during trials.

The time that the females spent in association with each of the two stimulus males was used to measure mate preference, as this is known to be a good indicator of mating behavior in captive birds (Clayton 1990; Witte 2006; Snowberg & Benkman 2007). Even if we are simply measuring affinity for association, this could lead to assortative mating between groups if females prefer to associate with males of their own familiar flock and assortative mating is a byproduct of such association (Clayton 1990). The method has been used a proxy for mate preference in many laboratory studies in several taxa (Enstrom 1993; Johnson *et al*. 1993; Nolan & Hill 2004; Cummings & Mollaghan 2006; Wong & Rosenthal 2006; Snowberg & Benkman 2007)

We conducted two separate experiments. In the first, we analyzed the relationship between female preference and familiarity (n= 15 trials). In this experiment females had to choose between a familiar and an unfamiliar male, both ornamented with a large yellow wing stripe. The time that the female spent with each of the two males was transformed into a percentage. We analyzed the data using a Wilcoxon signed-rank test (Neave & Worthington 1992). Our null hypothesis was that females would spend equal time with each of the two stimulus birds, against the alternative that females would spend more than 50% of the time with familiar males. We used different females for each trial (n=15 females), and a different pair of males (no male was repeated) for each female tested (n=30 males), thus ensuring independence. Females used in the different trials were randomly chosen. The sets of two males were also chosen at random but under the restriction that the difference in size of the yellow wing stripe between the two stimulus birds should be less than 0.5mm.

In the second experiment we analyzed the interaction between familiarity and ornamentation (n=15 trials). Females had to choose between a poorly ornamented familiar male (small yellow wing patch) and a more ornamented unfamiliar male (large yellow wing patch). Time spent by the female at each of the two cages was again transformed to a percentage, and analyzed using a Wilcoxon signed-rank test. We used different females for each trial (n=15 females) and a different pair of males (no male was repeated) for each female tested (n=30 males). The birds were the same as those used experiment one, but we ensured that they were confronted with different males to those used in experiment one. Females used in each trial were chosen at random. The sets of two males were also chosen at random but this time under the restriction that the difference in size of the yellow wing stripe between the two stimulus birds should be at least 2 mm, as this had previously proven sufficient to detect a preference by the females (Senar *et al.* 2005).

It should be noted that experiments one and two were not carried out sequentially; the order of each experiment for each female was chosen at random. We did not perform a control experiment because the experimental design was identical to that used in Senar *et al.* (2005).

Results

In the first experiment, where we presented siskin females with two males that were equally ornamented (i.e. with long yellow wing stripes) but different in social familiarity, females showed a significant preference for the familiar males (Wilcoxon signed-rank test, T=8.0, p<0.01, n=15 trials; figure 1).

In the second experiment, females had to choose between a poorly ornamented familiar male (small yellow wing patch) and an ornamented unfamiliar male (large yellow wing patch). In this experiment, female siskins did not show any significant preference (Wilcoxon signed-rank test, T=44.0, n.s., n=15 trials; figure 1).

Discussion

Previous studies analysing the interaction between male attractiveness and social familiarity in mate choice found familiarity bred contempt and that attractiveness was more important than familiarity when choosing a mate (Patricelli *et al*. 2003; Zajitschek *et al*. 2006; Zajitschek & Brooks 2008). The results from our study in siskins support a new view, in that attractiveness alone in some species is not enough and that social familiarity adds to attraction.
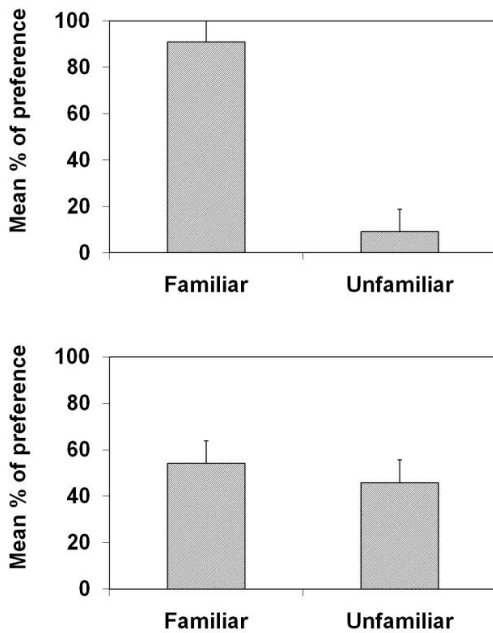
Figure 1. Results from the two experiments in which the siskin females were presented with a choice between two male siskins differing in social familiarity (Familiar and Unfamiliar) and sexual attractiveness, measured as the relative length of the yellow wing stripe. In the first experiment, females had to choose between a familiar and an unfamiliar male, both ornamented with a large yellow wing stripe (both attractive). In the second experiment, females had to choose between a poorly ornamented familiar male (small yellow wing patch) and a more ornamented unfamiliar male (large yellow wing patch). Results are expressed as the mean percentage (± S.E.) of time that the test female stayed with either stimulus male during a total of 4 hours (14.400 s.). The outcome of non-parametric Wilcoxon signed-rank tests is provided in the text (N = 15 trials for both experiments).

The familiarity factor may help us understand patterns of sympatric local adaptation and divergent ecological selection. This factor may be especially important in species in which different subpopulations cohabit during the mating period, since this cohabitation would otherwise provide ample opportunities for gene flow (Schluter *et al*. 2001; Newton 2008). Crossbills *Loxia* spp. are considered a typical example; studies have shown that different cryptic species may cohabit in the same geographical areas but that they seem to mate assortatively (Benkman 1993; Groth 1993; Parchman *et al*. 2006; Snowberg & Benkman 2007; Summers *et al*. 2007; Edelaar *et al*. 2008; Snowberg & Benkman 2009). The citril finch *Serinus citrinella* is another example. Different subpopulations, known to adapt locally and to differ in morphometrics and genetic structure (Senar *et al*. 2006; Förschler *et al*. 2011), are allopatric during the breeding season but intermix in the shared wintering areas where pairing takes place (Borras *et al*. 2010; Borras *et al*. 2011). Citril finch subpopulations,

however, remain differentiated (Senar *et al*. 2006; Förschler *et al*. 2011). The siskin is a third example. Two different subpopulations, residents and transients, have been described in this species (Senar *et al*. 1992). These two subpopulations intermix and cohabit during the winter pairing period (Senar & Copete 1990; Senar *et al*. 1992), but subpopulations consistently differ in morphometrics (Senar *et al*. 1994, 2001).

Subpopulations remain differentiated in crossbills, citril finches, and siskins, even though cohabitation of the different subpopulations during the pairing period would provide ample opportunities for gene-flow (Choudhury & Black 1994; Borras *et al*. 2011). Here, we stress that a mating preference for socially familiar individuals, as found in the present study, could nicely explain the maintenance of these instances of local differentiation (Choudhury & Black 1994; Borras *et al*. 2011).

Interspecific variation in the role of familiarity in mate choice has been mainly attributed to the probability of inbreeding (Cheetham *et al*. 2008). This view would explain why in species with a high probability of inbreeding, as for instance in promiscuous species, females generally avoid males that are familiar to them (Cheetham *et al*. 2008). However, we think that interspecific variation in the role of familiarity in mate choice could also be related to interspecific variation in the degree of stability in the composition of groups, which is in fact a prerequisite for social familiarity. Carduline finches, like crossbills, citril finches and siskins, breed in semi-colonies that have high social interaction among their members (Newton 1972; Glück 1980). The high social costs of flock switching enhance membership stability (Senar *et al*. 1990; Cristol 1995; Balph 1979; Mundinger 1979; Wilson 2006), and data suggest that flocks formed in the original breeding localities may even remain together in the wintering grounds (Wilson 2006). In this scenario, if birds develop a mating preference for familiar birds, flock structure would favor pairing with individuals from the same breeding subpopulation, thereby reducing gene-flow and allowing local adaptation.

There is increasing evidence that animals may use multiple criteria in mate choice (Candolin 2003). Our present results suggest that in some species, social familiarity adds to attractiveness. As preference for familiar individuals as mates could favor local adaptation, social familiarity should be regarded as an important selection pressure in modulating the evolution of characters.

References

Balph, M. H. 1979. Flock stability in relation to social dominance and agonistic behavior in wintering Dark-eyed Juncos. *Auk,* 96: 714-722.

Benkman, C. W. 1993. Adaptation to single resources and the evolution of crossbill (*Loxia*) diversity. *Ecol. monograph,* 63: 305-325.

Borras, A., J. Cabrera, X. Colome, T. Cabrera and J. C. Senar. 2010. Citril finches during the winter: patterns of distribution, the role of pines and implications for the conservation of the species. *Anim. Biodivers. Conserv.,* 33: 89-115.

Borras, A., J. Cabrera, X. Colome, T. Cabrera and J. C. Senar. 2011. Patterns of connectivity in the Citril finch: sympatric wintering of allopatric birds? *Bird Study,* in press.

Burley, N., G. Krantzberg and P. Radman. 1982. Influence of colour-banding on the conspecific preferences of zebra finches. *Anim. Behav.,* 30: 444-455.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biol. Rev.,* 78: 575-595.

Cheetham, S. A., M. D. Thom, R. J. Beynon and J. L. Hurst. 2008. The effect of familiarity on mate choice. *Chem. Signal,* 11: 271-280.

Choudhury, S. and J. M. Black. 1994. Barnacle geese preferentially pair with familiar associates from early-life. *Anim. Behav.,* 48: 81-88.

Clayton, N. S. 1990. Mate choice and pair formation in Timor and Australian mainland Zebra Finches. *Anim. Behav.,* 39: 474-480.

Cristol, D. A. 1995. Costs of switching social groups for dominant and subordinate dark-eyed juncos (*Junco hyemalis*). *Behav. Ecol. Sociobiol.,* 37: 93-101.

Cummings, M. and D. Mollaghan. 2006. Repeatability and consistency of female preference behaviours in a northern swordtail, *Xiphophorus nigrensis*. *Anim. Behav.,* 72: 217-224.

Edelaar, P., K. van Eerde and K. Terpstra. 2008. Is the nominate subspecies of the common crossbill *Loxia c. curvirostra* polytypic? II. Differentiation among vocal types in functional traits. *J. Avian Biol.,* 39: 108-115.

Enstrom, D. A. 1993. Female choice for age-specific plumage in the orchard oriole: implications for delayed plumage maturation. *Anim. Behav.,* 45: 435-442.

Förschler, M. I., J. C. Senar, A. Borras, J. Cabrera and M. Björklund. 2011. Gene flow and range expansion in a mountain-dwelling passerine with a fragmented distribution. *Biol. J. Linn. Soc.,* 103: 707-721.

Glück, E. 1980. Ernährung und Nahrungsstrategie des Stieglitzes *Carduelis carduelis*. *Ökol. Vögel (Ecol. Birds),* 2: 43-91.

Groth, J. G. 1993. Call matching and positive assortative mating in Red Crossbills. *Auk,* 110: 398-401.

Hill, G. E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Anim. Behav.,* 40: 563-572.

Johnson, A. M., G. Chappell, A. C. Price, F. H. Rodd, R. Olendorf and K. A. Hughes. 2010. Inbreeding depression and inbreeding Avoidance in a natural population of guppies (*Poecilia reticulata*). *Ethology,* 116: 448-457.

Johnson, K., R. Dalton and N. Burley. 1993. Preferences of female American goldfinches (*Carduelis tristis*) for natural and artificial male traits. *Behav. Ecol.,* 4: 138-143.

Kelley, J. L., J. A. Graves and A. E. Magurran. 1999. Familiarity breeds contempt in guppies. *Nature,* 401: 661-662.

Mank, J. E. 2009. Sexual selection and Darwin's mystery of mysteries. *Science,* 326: 1639-1640.

Mariette, M. M., S. R. K. Zajitschek, C. M. Garcia and R. C. Brooks. 2010. The effects of familiarity and group size on mating preferences in the guppy, Poecilia reticulata. *J. Evol. Biol.,* 23: 1772-1782.

Martin, A. 1994. A key to ageing siskins. *Ringers' Bull.,* 8: 76-77.

Mateos-Gonzalez, F., J. Quesada and J. C. Senar. 2011. Sexy birds are superior at solving a foraging problem. *Biol. Lett.*, 7: 668-669.

Mundinger, P. C. 1979. Call learning in the carduelinae: ethological and systematic considerations. *Syst. Zool.,* 28: 270-283.

Neave, H. R. and P. L. Worthington. 1992. *Distribution-Free Tests*, 2 edn. Routledge, London.

Newton, I. 1972. *Finches*. Collins, London.

Newton, I. 2008. *The Migration Ecology of Birds*. Academic Press, London.

Nolan, P. M. and G. E. Hill. 2004. Female choice for song characteristics in the house finch. *Anim. Behav.,* 67: 403-410.

Panhuis, T. M., R. Butlin, M. Zuk and T. Tregenza. 2001. Sexual selection and speciation. *Trend. Ecol. Evol.,* 16: 364-371.

Parchman, T. L., C. W. Benkman and S. C. Britch. 2006. Patterns of genetic variation in the adaptive radiation of New World crossbills (Aves: *Loxia*). *Mol. Ecol.,* 15: 1873-1887.

Patricelli, G. L., J. A. C. Uy and G. Borgia. 2003. Multiple male traits interact: attractive bower decorations facilitate attractive behavioural displays in satin bowerbirds. *Proc. R. Soc. Lond. B,* 270: 2389-2395.

Price, T. 2008. *Speciation in Birds*. Roberts and Company Publishers, Greenwood Village, Colorado, USA.

Schluter, D., J. W. Boughman and H. D. Rundle. 2001. Parallel speciation with allopatry. *Trend. Ecol. Evol.,* 16: 283-284.

Senar, J. C., A. Borras, J. Cabrera, A. Cabrera and M. Björklund. 2006. Local differentiation in the presence of gene flow in the citril finch *Serinus citrinella*. *Biol. Lett.,* 2: 85-87.

Senar, J. C., P. J. K. Burton and N. B. Metcalfe. 1992. Variation in the nomadic tendency of a wintering finch *Carduelis spinus* and its relationship with body condition. *Ornis Scand.,* 23: 63-72.

Senar, J. C., M. Camerino and N. B. Metcalfe. 1990. Familiarity breeds tolerance: the development of social stability in flocking Siskins (*Carduelis spinus*). *Ethology,* 85: 13-24.

Senar, J. C., M. Camerino and F. Uribe. 2001. Body mass regulation in resident and transient wintering siskins *Carduelis spinus*. *Etología,* 9: 47-52.

Senar, J. C. and J. L. Copete. 1990. Observación de alimentación de cortejo en Lúganos *Carduelis spinus* invernantes. *Butll. GCA,* 7: 11-12.

Senar, J. C., J. Domènech and M. Camerino. 2005. Female Siskins choose mate by the size of the yellow wing stripe. *Behav. Ecol. Sociobiol.,* 57: 445-469.

Senar, J. C. and D. Escobar. 2002. Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. *Avian Science,* 2: 19-24.

Senar, J. C., J. Lleonart and N. B. Metcalfe. 1994. Wing-shape variation between resident and transient wintering Siskins *Carduelis spinus*. *J. Avian Biol.,* 25: 50-54.

Snowberg, L. K. and C. W. Benkman. 2007. The role of marker traits in the assortative mating within red crossbills, *Loxia curvirostra* complex. *J. Evol. Biol.,* 20: 1924-1932.

Snowberg, L. K. and C. W. Benkman. 2009. Mate choice based on a key ecological performance trait. *J. Evol. Biol.,* 22: 762-769.

Summers, R. W., R. J. G. Dawson and R. E. Phillips. 2007. Assortative mating and patterns of inheritance indicate that the three crossbill taxa in Scotland are species. *J. Avian Biol.,* 38: 153-162.

Svensson, L. 1992. *Identification Guide to European Passerines*, 4 edn. L.Svensson, Stockholm.

van Doorn, G. S., P. Edelaar and F. J. Weissing. 2009. On the origin of species by natural and sexual selection. *Science,* 326: 1704-1707.

Wilson, W. H. J. 2006. Evidence of flock fidelity in Common Redpolls and Pine Siskins. *N. Am. Bird Bander,* 31: 105-109.

Witte, K. 2006. Time spent with a male is a good indicator of mate preference in female zebra finches. *Ethol. Ecol. Evol.,* 18: 195-204.

Wong, B. B. M. and G. G. Rosenthal. 2006. Female disdain for swords in a swordtail fish. *Am. Nat.,* 167: 136-140.

Zajitschek, S. R. K. and R. C. Brooks. 2008. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). *Am. Nat.,* 172: 843-854.

Zajitschek, S. R. K., J. P. Evans and R. Brooks. 2006. Independent effects of familiarity and mating preferences for ornamental traits on mating decisions in guppies. *Behav. Ecol.,* 17: 911-916.