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Sexual selection: the influence of personality, behavioural and ornamental traits in the mate choice of Serin (*Serinus serinus*)

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Paulo Gama Mota (Universidade de Coimbra)

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Abstract

In birds, the most common signals of sexual selection are plumage colouration and song. Female mate choice usually favours sexual signals that express male quality and condition. The expression of colourful plumage can signal individual quality, since its development is generally costly. Furthermore, some empirical works made in the last few years show that other behavioural traits, such as maintenance of the ornaments or activity patterns can also signal condition and can be used in the mate choice context.

This thesis intends to clarify the value of the visual signals of colouration, and its combination with maintenance behaviour and personality traits of Serin males in female preference. The Serin, *Serinus serinus*, is a songbird in which males sing complex songs and show a yellow colouration, more intense in the breast and crown.

A series of experiments were made to investigate (1) the importance of colouration, visible and UV wavelengths, in female preference (2) the role of plumage maintenance behaviour in male's mating behaviour (3) the presence of personality traits and its influence in mate choice context.

Our results show that male yellow carotenoid plumage colouration is sexually selected, as females are more responsive to this and that, when controlling for background, UV wavelength is a component that influences female mate choice. We also demonstrated that maintenance behaviour is performed during more time by the more colourful males so it's suggestive that maintenance is carried out to reinforce male's appearance. Regarding personality traits, it was found consistent differences in behaviour and its influence in mate choice context, as bolder and exploratory individuals were also more active during the trials in detriment of shy and inactive ones.

This thesis provides a broad view of how can different factors such as colouration, maintenance behaviour and personality traits influence the behaviour ecology of the serin, specifically the mate choice in this species.

Keywords: Mate choice; Colouration and UV; Preening: Personality; *Serinus serinus*

Resumo

Nas aves, os sinais mais comuns de selecção sexual são a coloração da plumagem e o canto. A escolha de par feminina normalmente favorece sinais que expressem qualidade e condição do macho. A expressão de coloração da plumagem dos machos pode sinalizar qualidade devido aos custos associados à sua produção. Para além disso, alguns trabalhos empíricos realizados no últimos anos mostram que outras características comportamentais, como a manutenção da plumagem e padrões de actividade podem também sinalizar condição e ser usadas em contexto de escolha de par.

Esta tese pretende esclarecer o valor dos sinais visuais de coloração, e sua combinação com comportamentos de manutenção de plumagem e traços de personalidade nos machos Milheirinha, e a sua influência na preferência feminina. A Milheirinha, *Serinus serinus*, é um passeriforme em que os machos cantam canções complexas e mostram uma coloração amarela, mais intensa no peito e coroa. Uma série de experiências foram realizadas para investigar (1) a importância da coloração, visível e UV na preferência feminina (2) o papel do comportamento de manutenção da plumagem no comportamento de corte dos machos (3) a presença de traços de personalidade e sua influência em contexto de escolha de par.

Os nossos resultados mostram que a coloração por carotenóides é sexualmente seleccionada, já que as fêmeas lhes são mais sensíveis e, que o comprimento de onda UV é uma componente que influencia a percepção geral da coloração. Também demonstrámos que o comportamento de manutenção da plumagem é realizado durante mais tempo pelos machos mais coloridos, sendo sugerido que a manutenção é realizada para reforçar o aspecto do macho. Em relação aos traços de personalidade, encontrámos diferenças consistentes em comportamento e sua influência nas respostas comportamentais em contexto da escolha de par, já que indivíduos exploratórios e "bold" foram também mais activos durante os testes de escolha de par, em detrimento dos indivíduos inactivos e "shy".

Esta tese fornece uma visão geral de como diferentes factores, tais como coloração, comportamento de manutenção da plumagem, e traços de personalidade, podem influenciar a ecologia comportamental da milheirinha, especificamente a escolha de par nesta espécie.

Keywords: Escolha de par; Coloração e UV; Preening: Personalidade; *Serinus serinus*

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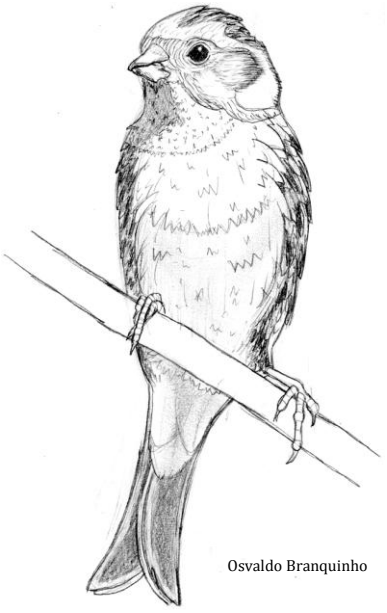
"In no aspect are birds so charming and entertaining as in their love affairs, far no creatures are so full of tender sentiment, and none display the said sentiment so gracefully, whether the display be a musical or spectacular one."

Frank Finn. 1907. Love among birds *In*: Ornithological & Other Oddities



Osvaldo Branquinho

Chapter 1 General Introduction

Evolution and Sexual selection

In *The Origin of Species* (1859), Charles Darwin described the processes of evolution of adaptive differences in species related with increase of survival. Readily, he realized the difficulty to explain how selection could act on phenotypic traits, like the peacock's tail, that reduces or is not easily correlated with survival. Darwin presented in *The Descent of Man and Selection in Relation to Sex* book(Darwin, 1871), an explanation for the evolution of sex differences and sexual traits. He proposed the concept of sexual selection, which he thought was different from natural selection, since it explained the phenotypic characters that did not confer advantage for the survival of the individuals that presented them but were essential to increase the reproductive success. He suggested a functional division of sexually dimorphism, the primary and secondary sexual traits: the first being linked with reproduction itself while the secondary sexual characters serve to attract and compete for a mate.

Darwin identified two mechanisms of sexual selection: competition between individuals from the same sex for a mate - intra-sexual selection - and the ability to attract the preference of the mate - inter-sexual selection, i.e., mate choice.

In intra-sexual selection, it's common to observe secondary sexual characteristics, especially in males, that limit the survival of the individuals, like the deer's antlers or the big claws of crabs. Nevertheless, in a competition context, the investment on those characteristics can confer the access to a mate (Krebs and Davies, 1993; Andersson, 1994). In the inter-sexual selection, females usually choose the males by the observation of elaborate characteristics (Krebs and Davies, 1993; Andersson, 1994).

Mechanisms of female preferences

Darwin's theory of sexual selection remained practically ignored until 100 years later (Mota, 2010). The most difficult part of this theory is to explain the evolution of female mate choice. In many cases the female's choice is very clear, and it is observable a direct benefit to the reproductive success, but still, there are various cases that is hard to recognize the advantage of mate selection (Andersson, 1994; Krebs and Davies, 1997). The

controversy and the difficulty to understand intersexual selection made this field one of the most explored research areas, experimentally and theoretically, seeking to explain the origin, function and evolutionary significance of elaborated male traits (also called ornaments), and the reason of female preference for these traits (Andersson, 1994). Several models were proposed to explain the evolution of female's preference, that can be generally divided in two types: Arbitrary preferences (Buchholz, 1995) and indicator models (Andersson, 1994).

The arbitrary preferences models theorize that a specific trait is not related to any fitness quality, but nonetheless is attractive to females. Fisher (1930) proposed for the first time a central model to future empirical studies, to explain Darwin's theory of mate choice, by the "runway" process. This theory explains that selection will favour females with a preference for an attractive trait, as their descendants will acquire this attribute and preference. In the end, the preference originates a positive feedback mechanism, as more attractive males will attract more females and the trait will be inherited by sons and the preference by daughters. In another perspective and more recently, Ryan (1990) proposed the "sensory bias" theory that explains the evolution of the sexual selected traits by a bias in the sensorial system of the females. The females are attracted to supernormal stimulus, i.e., they will choose ornaments that are different from the normal population.

The indicator models hypothesise that an ornament evolves in association with the genetic and phenotypic quality of the male, because the ornament is an honest indicator of quality (Andersson, 1994). In order to be honest, the ornament must entail costs that individuals of inferior quality cannot support. In this type of signalling system, Zahavi (1975) was the leading to propose the "Handicap" principle. He suggested that females tend to prefer male traits that are a handicap exactly because they advertise the ability to survive no matter what, and so they are indicators of condition and viability or a higher genetic quality, which indirectly benefit the female in reproductive success.

Unlike Handicap principle, Hamilton and Zuk (1982) suggested that mate-choice may be due to a process where females might select healthy males, by exerting choice on traits that signal the carrier's health status or resistance to disease. Specifically, Hamilton and Zuk describe that parasites can affect the attractiveness, and that the degree of resistance to the parasites is reflected in male ornaments, which can be used in mate choice. Females

prefer to mate with males who demonstrate their superior genetic quality to survive disease, obtaining viable genes to their offspring.

After the studies of Hamilton and Zuk where the male characteristics were described as signalling a resistance to parasite, the further studies began to focus in the immune system. In this process, Folstad and Karter (1992) postulated the Immunocompetence handicap hypothesis, where they suggest that the expression of secondary sexual characteristics can signal an immune capacity of the individual, due to a negative relation between immunity response and circulating androgens.

Despite the amount of theories and research evidence in this area, there is still a lot of work to be done to understand how and why mate choice evolved in each species.

How and why females choose

The role of choosing the partner and being chose is normally associated with the reproductive potential and costs, that is generally greater for females than for males (Andersson, 1994).

Mate choice is defined as a pattern of behaviours and sensory traits that influence the chooser and that make the signal carrier more probable to be chosen (Heisler I.L and others, 1987). The chooser partner, selects a mate on the basis of a direct ability like good resources or parental quality (Krebs and Davies, 1997) or by single or multiple indicators and different strategies to assess mate quality (condition and genetic quality). These indicators can include for e.g. male song (Collins, et al., 1994; Cardoso, et al., 2007; Byers and Kroodsma, 2009), territory quality (Bart and Earnst, 1999), colouration (Hill, 2006), symmetry in plumage, (Swaddle and Cuthill, 1994; Fiske and Amundsen, 1997), behavioural patterns (Godin and Dugatkin, 1996) and even cognitive traits (Boogert, et al., 2011). The effectiveness of a specific trait will be affected by the information content of the signal, the condition and the ability of the transmitter, the environmental context, the physiological condition of the receiver, its decision status and criteria, and the trade-off between costs and benefits for the decision (see Endler, 2000).

Mate searching it's an effort investment in evaluation of potential mates, influenced by received signals, the number of individuals sampled and the total time spend examining

each potential mate (Jennions and Petrie, 1997; Kokko and Wong, 2007). Also, the quality state of the female influences her choice (Jennions and Petrie, 1997), simultaneously with environmental factors like predation risk (Pocklington and Dill, 1995), spatial and temporal variation (territory and resources), time constraints concerning optimal period of reproductive success (Jennions and Petrie, 1997), and other environmental effects that influence discrimination of the signal. Social factors may also influence female's choice (Godin, et al., 2005) for e.g., previous experience, female phenotype and age (Kodric-Brown and Nicoletto, 2001), conspecific interactions like male-male eavesdropping (Doutrelant and McGregor, 2000), occurrence of an ample phenotypic variation (Griffith, 2000), female-female competition (Clutton-Brock, 2009), and others. In the end, if mate choice results in benefits that compensate the costs of the decision, selection will preserve these preferences.

Mate choice may drive selection on the preference itself and on male traits. If a significant change in the preference pattern occurs, it can alter the direction of the selection and even disturb sufficiently to lead to speciation (Higashi, et al., 1999). Moreover, understanding mating preferences can provide a greater knowledge of inter-specific differences and even evolutionary history of speciation process.

Sexual traits

In birds, the most common sexually selected signals are colouration and song (Halliday, 1983; Andersson, 1994). However, some empirical work made in the last few years shows that some behavioural traits can also be used in mate choice (Gail, et al., 2003; Griggio, et al., 2010a; Boogert, et al., 2011). Courtship behaviour involves the emission of several signals that, if correspondent to an honest message, must have a cost associated with their production, contributing to a direct or indirect benefit to the female and her offspring (Andersson, 1994; Bradbury and Vehrencamp, 1998).

- Colouration

Colourful displays were one of the bases for the formulation of sexual selection theory by Darwin. But it was only in 1939, that Noble and Curtis confirmed for the first time, with jellyfish, that colour displays are preferred by females.

It is now known that the expression of conspicuous and colourful plumage, bills and crests, are in many species honest signals of individual quality, since its development is generally costly and condition dependent (Andersson, 1994; Delhey, et al., 2007).

Sexual dichromatism – where one sex exhibits a noticeable visual signal in relation to the other - is common in many species (Andersson, 1994; Hill, 2006). The conspicuous colouration explores the sensory system of the receptor (Endler, 1990), together with ambient light, the contrast of the background (Bradbury and Vehrencamp, 1998), the size and the shape of the colouration area (Endler and Mielke, 2005), and the colour of additional structures (crests, tails, etc.). Colour signalling works if the signal reaches effectively its target to exhibit its content, otherwise the value of the content loses its importance (Andersson, 2000).

Carotenoids

The colouration may be due to the expression of various types of pigments, the most common are melanin and carotenoids, but it is also possible to be due to the presence of rare pigments like porphytin, pterin, flavin and others (McGraw, 2006). The plumage of the species studied, *Serinus serinus*, results from pigmentation by carotenoids (Stradi, et al., 1995). Carotenoids are pigments components of the colours red, yellow and orange (MacGraw, 2006), which concentrations are directly correlated with measurements of saturation and hue (Saks, et al., 2003). Birds can deposit the pigments in the fat, skin or in adjacent structures like feathers (Olson and Owens, 1998).

The first experimental evidence for mate preferences linked to carotenoid expression was with the house finches (Hill, 1990), where females preferred males with redder plumage. Later, several experimental studies with american goldfinches, (Johnson, et al.,

1993), yellowhammers (Sundberg, 1995) and red jungle fowl (Zuk, et al., 1990), confirmed that females preferred males with high presence of carotenoid pigmentation in sexual traits.

The birds, and other vertebrates, are not capable to synthesize carotenoids, obtaining it through diet (Goodwin, 1984). The most well-known case is the Flamingo, which gains a more intense pink/orange carotenoid-colour depending on their carotenoid rich diet (Fox, 1962), based on algae and crustaceans. In fact, it has been demonstrated in several controlled studies and a few field work (see Hill, 1999) that changing access to carotenoid pigments during moult can determine plumage colouration. It is clear the costs associated with this type of colouration since carotenoid availability can be a limiting factor (Blount, 2004; MacGraw, 2006). Female mate choice can be based on preference of carotenoid-based plumage for foraging abilities (Hill, 1990) or even nutritional condition at the time of moult (Hill and Montgomerie, 1994).

Other factors can affect carotenoid expression. Carotenoid molecules have an important role as anti-oxidative and immune-stimulatory (Olson and Owens, 1998; Blount, et al., 2003). Recently it was proposed that a possible trade-off could exist between the pigmentation of the feathers and the presence of carotenoids in another function of the body when confronted with an infection (Blount, 2004). Individuals with poor quality will allocate more carotenoids to immune defence and individuals with better condition will have carotenoids available to the signalling traits that advertise their superior quality (Blount, 2004).

Carotenoids are also under the influence of hormonal factors. The expression of sexual traits are frequently controlled by sex-steroid hormones like testosterone (Mougeot, et al., 2007). Testosterone possibly can modulate expression by allocating carotenoids to ornaments (Folstad and Karter, 1992), an hypothesis that is been testing now with serins.

Parasites

It is also possible that females assess colouration for parasite resistance. Several studies with birds showed a negative effect of parasites in the expression of colouration (Mougeot, et al., 2007) and consequently in mate preference (Buchholz, 1995; Brawner and

Hill, 1999). In American goldfinches (Olson, 1996; McGraw and Hill, 2000), endoparasites that affect the uptake of dietary components, reduce carotenoid-based colouration in plumage. Ectoparasites can also affect carotenoid display in integument (Blount, 2004). Thus, variation in the colour expression can indicate an immune capacity to fight against parasites.

Avian vision and UV colouration

The perceived signal always depends on sensorial capacities of the receiver of the signal (Endler, 2000). In recent years the role of colour perception and the mechanisms of its production have received great attention, since the discovery of the specific properties of avian visual system. Birds, specifically passerines, have a greater capacity for colour perception in comparison to humans, as they can see the entire human-visible spectrum (400-700 nm) plus ultraviolet spectrum (320-400 nm). Ultraviolet vision was firstly recognized in studies with hummingbirds and their foraging behaviour (Huth and Burkhardt, 1972) and an experimental study with pigeons (Wright, 1972) while the first avian UV visual pigment was detected in 1993 (Maier and Bowmaker, 1993).

Birds have tetrachromatic vision, due to the existence of four types of cones (Maier and Bowmaker, 1993), that are sensitive to long-wave or red (LWS; λmax 543–571 nm), medium-wave or green (MWS; λmax 497–510 nm), short-wave or blue (SWS; λmax 430–463 nm), and ultraviolet (UVS; λmax 320-420 nm) (Cuthill, et al., 2000a; Cuthill, 2006) (Figure 1).

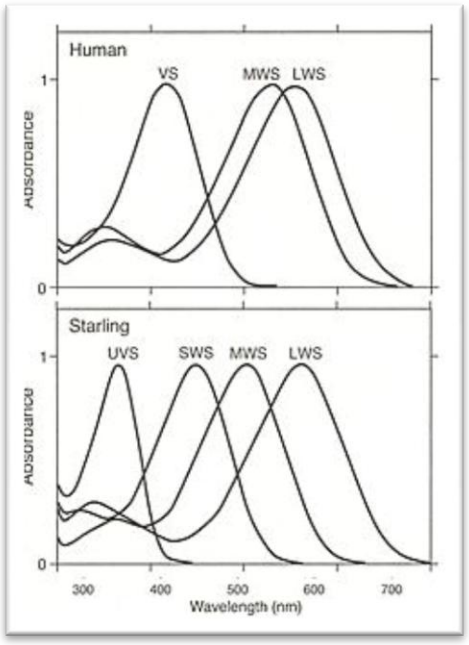


Figure 1 –Comparison between human and starling colour vision. Humans are trichromatic, with three types of cone cells, each of them with a different photosensitive pigment, that correspond to long, medium and short wavelength. Starlings (birds) are tetrachromatic, having one more cone cell corresponding to UV wavelength. Image from Cuthill (2006).

Thus, we cannot rely on the human eye in order to measure colouration on birds, as we could only perceive part of the totality of birds' visual spectra. Specific techniques, such as a reflectance spectrophotometry (Endler, 1990; Cuthill, et al., 1999), are then needed to obtain accurate results.

Many birds also have evolved mechanism in the visual pigments to confer light filtering - coloured oil droplets. Oil droplets change the spectral sensitivity by reducing overlapping in spectrum and thus increasing colour discrimination (Vorobyev, 2003).

The serin has a yellow plumage visible from green to red and a smaller peak in the ultraviolet region (Figure 2 in Chapter 2), typical of carotenoid pigmentation plumage (MacDougall and Montgomerie, 2003). UV reflectance in birds can be caused and modified by structural colours of the feathers (Griggio, et al., 2010b) pigment deposition (Bleiweiss, 2005), external influences like soiling (Zampiga, et al., 2004), and possibly the application of uropygial oil (Piersma, et al., 1999).

Several experimental studies have shown that of UV is important in mate choice (Bennett, et al., 1996; Andersson and Amundsen, 1997; Bennett, et al., 1997; Hunt, et al., 1997; Hunt, et al., 1998; Pearn, et al., 2001; Siitari and Huhta, 2002). In a study with bluethroats (Andersson and Amundsen, 1997), females preferred males that reflected UV than males whose UV reflection was reduced. This was also seen in studies with zebra finches (Bennett, et al., 1996), and with starlings (Bennett, et al., 1997). In wild pied flycatcher (Sirkiä and Laaksonen, 2009), males that reflected UV had an elevated pairing success, in detriment of the ones that UV was reduced, but only early in the season.

It was suggested that UV may have a special role in communication among birds (Guilford and Harvey, 1998). This issue is controversial, because although some works indicate evidences for the importance of UV signals in mate choice, most of the works didn't investigate methodically the importance of different parts of the avian visual spectrum (Stevens and Cuthill, 2007).

The most widely accepted way to test if UV reflection plays a role in mate choice is by experimental manipulation of the perceived wavelength (see Cuthill, et al., 2000a). There are two common ways to do it: using filters to block ultraviolet light (Maier, 1993; Hunt, et al., 1997) or application of UV-blocking chemicals (Andersson and Amundsen, 1997; Johnsen, et al., 1998), both with their limitations. By using filters, besides altering the subject it also modifies the environment i.e. the background and its light environment. The use of direct chemical application is more selective but can modify plumage physical properties and behaviour (Cuthill, et al., 2000a). Nonetheless, both methods showed similar results, as by blocking UV-light it can reduce attractiveness of the individual manipulated (review by Cuthill, et al., 2000a).

In a previous work with Serins, mate choice tests were made in an indoor apparatus with more and less colourful males of a natural range, where UV wavelength was blocked with a UV-blocking filter placed in front of the more colourful male compartment (Monteiro, 2008). Females showed a preference for males with more intense colouration only when the UV-filter was not present. When UV wavelength was blocked females no longer showed preference. However, by the method applied, the entire environment (the male and the background) changed appearance and this can influence female choice as the entire male's compartment became homogeneous or even too unnatural to the receptor of

the signal. In the present work we wanted to control for the background effect, so we used a more selective technique, a UV-blocking chemical that blocked the UV only in the male's ornament.

- Maintenance of ornaments

Ornamental traits require time and energy to maintain them. Birds spend 9.2 % of the day in maintenance behaviours, being grooming (preening and scratching) the main activity (92.6%) (Cotgreave and Clayton, 1994). Maintenance behaviour, in particularly preening, is recognized to be essential in bird self-cleaning, protection, feather care (Clayton, 1991; Cotgreave and Clayton, 1994) and removal of ectoparasites (Kulkarni and Heeb, 2007).

The uropygial gland is an indispensable part of preening behaviour and is present in most bird species, located in the tail end of the body. The secretion produced by this gland consists of a complex mixture of waxes that helps preserving the feather structure, delay feather wear (Moreno-Rueda, 2011) and add more water-proof to feathers (Jacob and Ziswiler, 1982), protects feathers against fungi and bacteria (Kulkarni and Heeb, 2007; Møller, et al., 2010), can help in odour communication (Jacob, et al., 1979) or even be used as a cosmetic colouration (Piersma, et al., 1999; Amat, et al., 2010; López-Rull, et al., 2010).

There is a possible trade-off between the exhibition of a well-maintained plumage and other important activities (like feeding and vigilance) and therefore maintenance behaviour can have a positive effect on signalling in mate choice (Walther and Clayton, 2005). In budgerigars, females chose cleaned males in detriment of soiled males, suggesting that females discriminate males that preen (Zampiga, et al., 2004) and that UV colours, related to preening, can express some sort of bird condition (Griggio, et al., 2010a). A recent study in domestic canaries (Lenouvel, et al., 2009) suggests that some males can be more efficient in performing grooming and consequently, clean the feathers better than others.

Cosmetic colouration

In 28 species of 13 bird families it has been reported the use cosmetic substances (Delhey, et al., 2008), that can either be produced by the animal itself or can be obtained from the environment. Most of the colours of the birds are acquired during moult, but it is possible that colouration is not a static trait as it was previously thought (Delhey, et al., 2008).

The application of cosmetic substances that modify the colour (visible and UV), has been hypothesised to enhance colouration and possibly play an important function in mate choice (Delhey, et al., 2007; Amat, et al., 2010; López-Rull, et al., 2010). It has been suggested that birds can modify plumage colouration by applying uropygial oil through preening (Piersma, et al., 1999; Delhey, et al., 2007; Amat, et al., 2010).

There are just a few studies in this area and the first to find carotenoids in the uropygial gland was just in 2010, in Flamingos *Phoenicopterus roseus* (Amat, et al., 2010). The authors show that carotenoids which are present in feathers are also present in preen oils, which act as a cosmetic in the plumage since they can change the appearance of individuals. The intensity of the colouration increases with the quantity of carotenoid applied (Amat, et al., 2010).

- Song

Many bird species produce songs that are used for communication within and between the sexes. In mate choice, preference for a particular song, can contribute to a direct or indirect advantage for the female and her offspring (Andersson, 1994; Bradbury and Vehrencamp, 1998). In Serins, the songs display a rapid succession of sounds and a wide frequency range, being composed by complex elements (Cardoso and Mota, 2007). In this species, male song is under sexual selection for higher frequency songs (Cardoso, et al., 2007). It can also be important in reproduction as it used for mate guarding and stimulation of the female for nest building (Mota, 1999; Mota and Hoi-Leitner, 2003).

- Personality

Animal species can demonstrate individual behavioural characteristics that are consistent over different circumstances, also known as personality traits (Réale, et al., 2010a), behavioural syndromes (Garamszegi, et al., 2009), temperaments (Réale, et al., 2007) and copying styles (Coppens, et al., 2010). In a broad definition (Réale, et al., 2007), animal personality refers to the behavioural differences between individuals (which can be extended to the family level, population or species), that are consistent over time and in different contexts.

The study of personality is important as it limits the behavioural plasticity, may explain maladaptive behaviour and the individual variation in behaviour (Sih, et al., 2004). Its influence on the ecology and evolution of species (Réale, et al., 2007) increases the importance of studies on the role of personality variation within and between species.

Behavioural ecologists ignored this issue until recently (Réale, et al., 2007). With the increase number of studies developed in the last decade (Réale, et al., 2010b), a considerable number of questions were raised, as it is still unknown the evolutionary origin of personality and how it is maintained. Several hypotheses were proposed, but sexual selection has been scarcely referred as a possible influence on personality traits. It is possible that sexual selection plays an important role in the origin and maintenance of personality traits, together with other evolutionary processes (Schuett, et al., 2010).

A few works have tried to relate sexually selected signals or even mate choice with personality traits. To my knowledge, Godin and Dugatkin (1996) showed for the first time a direct preference for a personality trait in guppies. Male conspicuous colours were correlated positively with boldness level, and females preferred these ones. But when given the opportunity to observe previously the male's behaviour, females preferred bolder males independently to their colouration. In another study with fighting fish, females preferred to mate with males that they have seen winning an aggressive interaction (Doutrelant and McGregor, 2000). In great tits (Naguib, et al., 2010), it was found that exploratory activity (considered a personality trait) was linked with singing activity (number of songs) of males, a sexual trait normally related to reproductive success.

Testing sexual selection theory - Mate Choice in the Laboratory

When the aim is to understand mate choice preferences, field experiments are difficult to identify and control for multiple features that can have correlated effects. The only possibility to separate different cues is through manipulation, which is very difficult to achieve in the field.

In laboratory it is possible, with a good experimental design, to dissociate the several and possible cues that influence mate choice and to understand their meaning. When performing mate choice tests, the principle is to separate females physically from males, with the purpose to eliminate habituation factors, reset their possible previous choice, and stimulate for mate choice. In this way, when doing a mate choice, a predictive of female's choice can be time spent by female in association with a male (Hill, 1990). A more direct preference is the observation of copulation solicitation (Jennions and Petrie, 1997) and/or construction of nests (Hill, 2006).

The negative aspect of laboratory experiments is that animals are taken from their natural habitat, and in a decision making scenario it is possible that the unnatural environment inhibit a result. Also it is difficult to examine the fitness consequence of female's choice. The ideal scenario is to have data from both field and laboratory.

Study species

Serinus serinus (Linnaeus, 1766) is a passerine, a cardueline finch, belonging to the family Fringillidae, genus Serinus, commonly used in behavioural studies, either in laboratory or in field.

The Serin is a bird of about 11.5 cm long, conical and short beak, forked tail. The species shows sexual dimorphism mainly on the plumage. Males and females have the same colouration and stripes in the plumage, but the female has less intense yellow but more intense brown stripes, and the male has more intense yellow colour in the forehead, breast and bib (Cramp, 1998).

Social features and reproductive behaviour

Outside the breeding season, late summer to February, the serin is a gregarious bird forming small flocks for feeding or migration (Newton, 1972; Cramp, 1998). When approaching breeding season, the males become more aggressive among them and start to sing enthusiastically further apart the group. The male finds a possible mate by courting the female while in the flock and only after pair-formation, they decide where to nest (Newton, 1972). The serin reproduce in neighbourhood groups while individuals can forage away alone or in flocks (Newton, 1972; Cramp, 1998). It is a monogamous species, with evidence of mate guarding to prevent extra-pair copulations (Mota and Hoi-Leitner, 2003).

In the south of the distribution range, the breeding and nesting period occurs from late February until July (Mota, 1995). The serin couple can perform several breeding attempts and produce several broods. Females lay 4 eggs (rarely 5) in consecutive days. Incubation is performed by the female and lasts 12 to 14 days and the young can stay in the nest for 14 to 16 days (Newton, 1972; Mota, 1995). Nests are heavily predated (Mota, 1995) so that one pair usually only raises one brood per season.

Food

The food consists mainly of seeds and other plant parts, such as *Ulmus*, *Betula*, *Taraxacum* in the spring, *Capsella*, *Polygonum* in summer and autumn and *Artemisia vulgaris* seed in the winter (Eber 1956 *in* Newton 1972) which are found in soil and shrubbery or near this areas or in open areas and semi-open. They can also eat invertebrates (Cramp, 1998).

Geographical Distribution and Habitat

The serin is a bird native of southern Europe, Asia Minor and northwest Africa, having become, after its recent expansion, particularly common in Europe (Olson, 1971) (Fig 2).

Its natural habitat is open forest areas of southern Europe, being now settled in a variety of habitats such as gardens, cultivated land with olives and cork oaks and in urban

areas (around villages) (Olson, 1971; Newton, 1972; Cramp, 1998). It has a very wide distribution in Portugal being one of the most common species of our breeding avifauna.

Conservation status

The Serin, according to the Red Book of Endangered Species of UICN (2011), is classified as Least Concern (LC), for being widely distributed throughout Europe and for its high population size.

Objectives for the thesis

This study intended to clarify the value of several behavioural and ornamental traits of male *Serinus serinus* that may influence mate choice in serins. The main objectives are:

(1) Investigate the importance of colouration, visible and UV wavelength, in female mate choice, by applying a selective technique that controls for background.

(2) Clarify the role of plumage maintenance behaviour, specifically preening, in mate choice in serins.

(3) Investigate the presence of personality traits and its influence in mate choice context.

Detailed hypothesis are described in each chapter.

Chapter 2 Meaning of colouration and UV reflectance in mate choice of Serin

Introduction

Female mating preference is predicted to be an important driving force in evolution and maintenance of secondary sexual traits (Darwin, 1871; Andersson, 1994). Most of the mechanisms of sexual selection that explain the evolution of intersexual systems in relation to female choice, predict that females should prefer characteristics that are reliable of male quality (Zahavi, 1975; Hamilton and Zuk, 1982; Andersson, 1994). The conspicuous ornamental colouration exhibited by males of many bird species as been demonstrated to be costly (Hill and Montgomerie, 1994), being a favourable quality indicator of males (Andersson, 1994).

Recently, avian colour vision and colouration has received a great attention, particularly the significance of ultraviolet (UV) plumage, that most birds can perceive (Cuthill, et al., 2000a). This is due to the existence of four visual cones cells, which are sensitive from the 320 to 700 nm, (Cuthill, et al., 2000a; Cuthill, 2006), and oil droplets that changes the spectral sensitivity by increasing colour discrimination (Vorobyev, 2003).

The capacity of many bird species to perceive UV light and reflectance on ornamental traits suggest that sexual selection operates also in these wavelengths. In fact, there is a substantial body of research into the influence of ultraviolet wavelengths in bird mate choice (Bennett, et al., 1996; Andersson and Amundsen, 1997; Bennett, et al., 1997; Hunt, et al., 1997; Hunt, et al., 1998; Pearn, et al., 2001; Siitari and Huhta, 2002). In all studies, females preferred males reflecting a complete spectrum over males whose UV reflection was reduced. Nevertheless, the function of UV wavelengths remains controversial, because although some ranges are of particular importance, there is insufficient evidence that the UV plays a major role in mate choice, as most of the facts point to UV be as important as other components of the spectrum (Stevens and Cuthill, 2007).

Previous studies with *Serinus serinus* (Monteiro, 2008), which measured the importance of colour in mate choice, revealed that females prefer males with more intense saturated yellow and in the absence of UV wavelength females didn't exhibit any preference, which could indicate that the UV is a key component of the colour of the males in this species. But, the absence of preference could be due to the unnatural environment created by the method applied – UV filters – that changed the appearance of the male and the background. Also it could affect male's behaviour as the filter was bi-directional, so the

male also perceived the female in a different way. In the present work, we used a more selective technique, a UV-blocking chemical that blocked the UV only in the male's ornament, and so controls for the background effect and female's appearance to the male.

In this study we seek to understand whether the colour and ultraviolet spectrum of the carotenoid plumage of the males are the basis of female sexual choices in serin. The serin (*Serinus serinus*) is adequate to this kind of study since the male plumage reflects in the UV and Visible spectrum, common in carotenoid-based plumages (Saks, et al., 2003) (Figure 2). The serin it's a small Fringillidae that shows sexual dichromatism resulting from carotenoid colouration: males have a yellow throat, breast, abdomen and crown but not females.

The main goals are (1) Test the preference of females for more colourful males without manipulating colour (2) Test the importance of the UV component of colour spectrum in female choice.

Material & Methods

The experiment was carried out between January and May 2011, in the Laboratory of Ethology of the University of Coimbra, Portugal. Birds were captured using mistnets and transported immediately to an indoor aviary in the Laboratory. During the experiments, the birds were kept in groups cages in the aviary of the Department of Life Sciences, and released in the end. The individuals were housed separated according to sex, visually isolated from each other and kept at room temperature with natural and regulated photoperiod. Birds had access *ad libitum* to a commercial mixture (European Finches Prestige, Versele-Laga) and tap water.

Identification, Morphometry and Parasites

Each bird was banded with numbered plastic black rings (A. C. Hughes) for subsequent individual identification. Age (one year or older) and sex was determined, as described by Svensson (1992). We also took several morphometric measurements such as standard weight with a pesola, (± 0.5 g), length of wing with a ruler (± 1mm), tarsus length, tail length and length, width and depth of the beak all measured with calliper (± 0.01mm)

(Appendix II - A). The number of ectoparasites was also quantified as described by Behnke et al. (1995) (Appendix I - B).

Colour assessment

The colour reflection of male serins was measured in February and repeated in April of 2011, using a spectrophotometer Ocean Optics USB4000 (Ocean Optics, Dunedin, FL, USA), with two lamps emitting deuterium and halogen (Mikropack Mini-DT-2-GS, UV-VIS-NIR Lightsource), that emitted light between 300nm and 700nm. Measurements were taken with a probe (optical cable OceanOptics R400-7 UV/VIS). The probe was attached to a rigid rubber protection that excluded external light and kept the probe at a fixed distance of 3mm and perpendicular to the surface of the feathers. The sampled area was of 28 mm^2 approximately. All measurements of the spectrum are expressed in the proportion of light relative to a white standard (Ocean Optics, WS-1-SS White Standard). Three random measurements of the four areas of yellow, outlined above, were made and the average was considered to the analysis. The values resulted from spectrophotometry analysis were used to calculate colour variables, according to Montgomerie (2006) adapted from Cardoso and Mota (2008) (formulas presented in Appendix I - C): full brightness, UV brightness, hue, saturation and saturation total UV.

We also measured the extent of yellow area in the crown and breast of males by overlapping a grid and counting squares on those areas.

Matching pairs

To conduct the experiments of mate choice, it was necessary to classify the males by their colour in order to control and manipulate the variables. For this purpose, we performed a Principal Component Analysis (PCA) with a set of measurements obtained from areas where the species have a colour based on carotenoids (Appendix II - C). We did not include the variables of colour (hue), as their coefficients of variation are almost null and the variables extension of the crown and breast, as well as all the colour variables of crown since they behaved differently, in the preliminary analysis, from all other variables.

The first factor of the PCA (PC1) explained 36.4% of the variation and discriminated brightness and saturation, both variables negatively correlated (Appendix II – C). PC1 was used to rank males considering saturation, being the most colourful males the ones with highly saturated ornamental plumage colouration. Based on the negative and positive values of PC1, we separated the males in two groups: the most colourful (M +) and less colourful (M-) (Appendix II – C). For the mate choice tests, pairs of males were formed by choosing one from each group, trying to maximize the differences in plumage colouration (Figure 2 for an example of the spectrum).

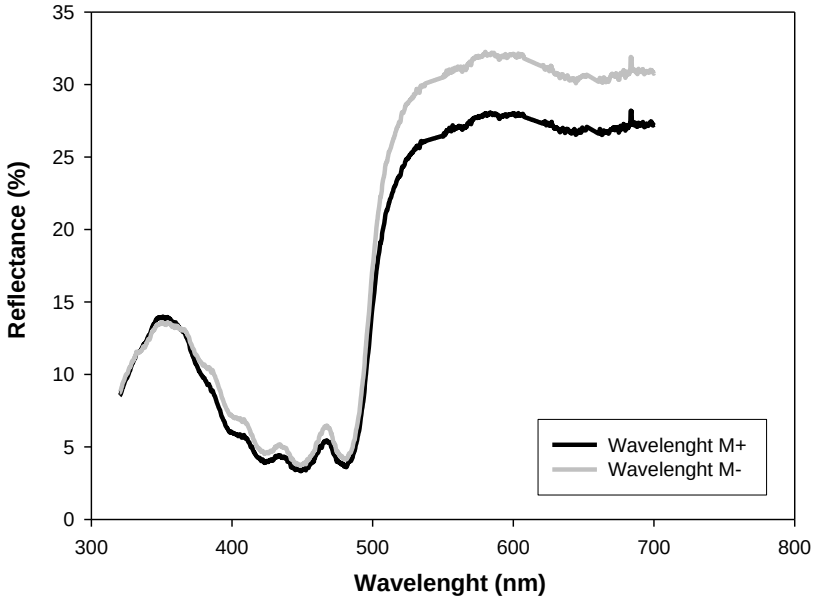


Figure 2 – Spectrogram of a more colourful (M+) and less colourful (M-) male pair, matched by the first score of the PCA and used in the mate choice test (M+: male 142; M-: male 180)

Experimental design

We performed a total of 19 successful mate choice tests in each trial (38 in total) (see appendix II - E). In each of these tests, a new female was used and a total of 17 males were presented to the females as stimulus birds. Males that were used more than once were matched to prevent repetition of pairs. The trials consisted in two parts: the first (Experiment I) without manipulation of the UV, and second (Experiment II) with manipulation of UV reflected by the plumage of the males. The same pairs of males were

used in both trials and they were presented to the same female, but changing their relative position to the female.

In Experiment I, we assessed the normal preference of the female. In the Experiment II, we carried a similar procedure used successfully by Andersson & Amundsen (1997), reviewed by Hill (2006), by blocking the UV transmission spectra of the more colourful male, and applying a neutral mixture in the less colourful male as a control. The protocol applied (Appendix II – D - Protocol 2) took into account the results of persistency and temporal variation of the treatment from Korsten and colleagues (2007). The UV treatment consisted in applying a chemical (Appendix II – D - Protocol 1) directly in the feathers, composed by a 75/25% mixture of duck preen gland oil (CDC, Avonmore Tackle Products, Rathdrum, Wicklow, Ireland) and an UV-absorbing chemical (Eusolex 9020 ®, Merck SA, Lisboa, Portugal). The neutral treatment was composed only with duck preen oil (CDC), to act as a control since it slightly reduced reflectance in 400-700 nm range as the UV treatment and did not affect the range between 300-400 nm. The UV Treatment effect was checked by spectrophotometry, as illustrated in Figure 3. Both UV and Neutral treatment differed only in UV colour (Figure 3).

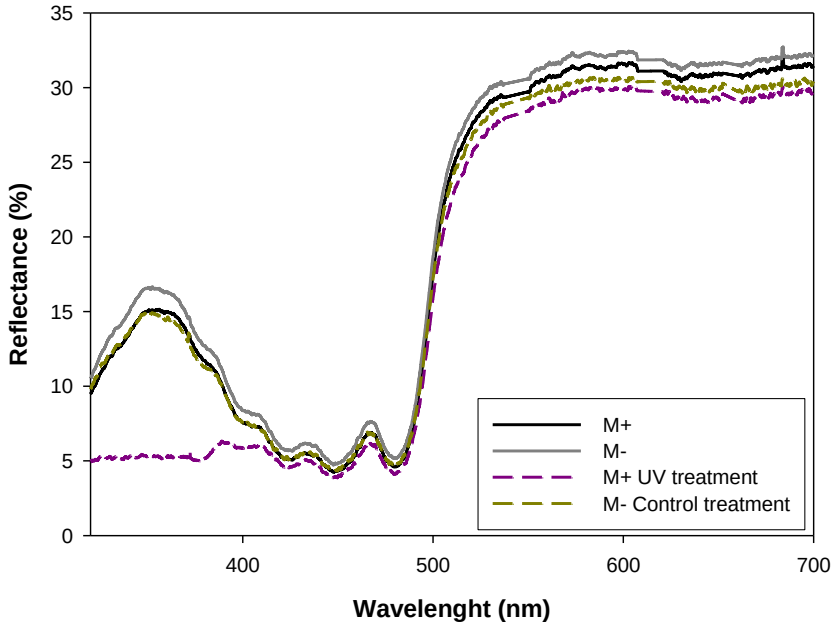


Figure 3. Spectral reflectance before and after the UV and Control treatment for 17 males. Each spectrum is in turn of averages of all the males, for all the coloured zones and the three measurements.

The mate choice tests were ran in a three-compartment indoor aviary (Appendix I - D), illuminated by fluorescent lamps with an emission spectrum similar to sunlight.

During each trial, the males were kept in separate compartments and didn't have visual contact with each other and the female was only separated by glass from both males. The adjacent areas to each male were designated as the "response areas", where we used time spent by females, in this interaction area, as a measurement of female preference (Hill, 1990). In these areas there were perches, so we could be able to register other behaviours of interest such as billing and hopping (complete Ethogram in Appendix I).

The tests were digitally recorded to a workstation using a video camera Sony SSC-DC378P and the sound was recorded using a Marantz PMD660 solid state and two microphones located in each compartments of the males.

All tests were performed in the morning to avoid behavioural changes related to time of the day. The tests lasted for 1 hour, being the first 30 minutes considered acclimatization time to the new place and the following 30 minutes were considered for recording behaviours. The tests were considered valid for analysis if they respected the following criteria: a) During the period of habituation, the first 30 minutes, the female visited the two males; b) During the test, the female stayed at least 10 minutes in the "response areas". The tests that did not comply with these requirements were not considered valid, and were repeated up to three times until they were valid or permanently excluded.

Behavioural and song analysis

The behaviour of the individuals were analysed using a computer with the software Observer ® XT (Noldus Information Technology) with help of an X-keys Jog & Shuttle. From the second half hour of the test we measured the following variables in both sexes: "Response Area" (Duration of visits to response area), "Perch" (frequency of visits to perch); "Billing" (frequency of billing), "Shock" (frequency of shocks in the glass).

Due to some problems in the video acquisition, we only analysed a total of 24 tests (12 in each trial).

Singing activity was practically inexistent during the trials, with just existence of normal vocalizations. It was thus excluded for further analysis and discussion

Statistical analysis

The statistical analysis was performed using software IBM SPSS Statistics ® 19.0 for Windows. We analysed the mate choice in females in both experiments, the differences in male's behaviour during each trial and the effect of the experiment on the activity of individuals. To check the distribution of the data we tested the variables by One-sample Kolmogorov-Smirnov test, and as most of our data were normally distributed we used parametric statistics in the analysis. When normality was not met, even after transformation, we used non-parametric statistics.

For mate choice, in Experiments I and II, we conducted a paired sample t-test to check whether there were differences between female's behaviour toward males. In Experiment II, the variable shock was eliminated from this analysis as the mean was near to zero (0) in both types of male (M+ UV and M – ctrl).

To check for any experimental effect in the individuals between the two trials we made a paired t-test to compare if there were differences between the first and the second trial, in males and in females.

To analyse a possible effect of morphometry and parasites in mate choice, we performed a one-way ANOVA, to examine possible differences between more colourful and less colourful males.

To check for any relationship between colouration, morphometry and parasites in males, we performed Pearson bivariate correlations.

In all tests the level of statistical significance was 0.05.

Results

Experiment I

Females spent significantly more time in the response area of M+ ($p<0.001$, Table 1) then in the in area of M- (Figure 4). They also visited more often perches, made more shocks, and done more billing forward to M+, but the difference was not significant in these variables (Figure 5 and Table I).

Table I. Comparison between female behaviour in response to males in Experiment I, by t-test for paired samples (F – female, M+ - more colourful male, M- - less colourful male, resp – time in the response area, perch – frequency of visits to the perch, bill – frequency of billings, shock – frequency of shocks in the glass. Test: t – value of the test, p – value of the probability, N=12, asterisk indicate significance).

	M+		M -			
	Mean	Std error mean	Mean	Std error mean	t	p
F resp	1092.74	42.37	607.51	43.97	5.772	0.000 *
F perch	28.00	8.13	20.25	6.59	2.157	0.054
F bill	15.33	3.15	10.25	2.21	1.561	0.147
F shock	1.83	0.716	1.08	0.49	1.110	0.291

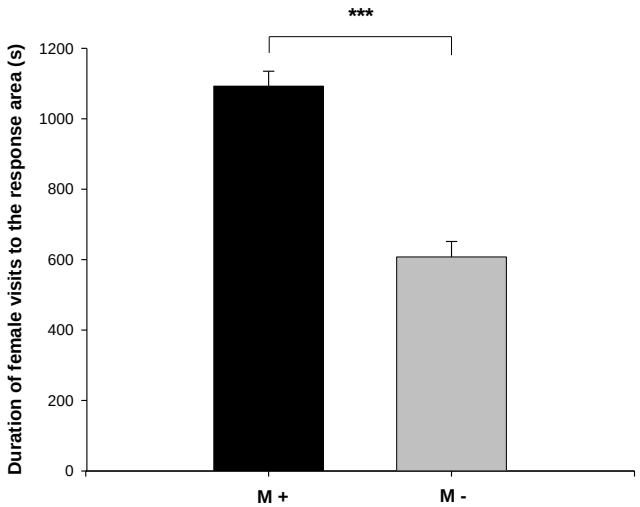


Figure 4. Duration of female visits to the response area (seconds) of more colourful males (M+) and less colourful males (M-), in the experiment I. The results are described as mean ± SEM and the asterisks on bars indicate significant differences in the overall average (n = 12), *** P <0.0001.

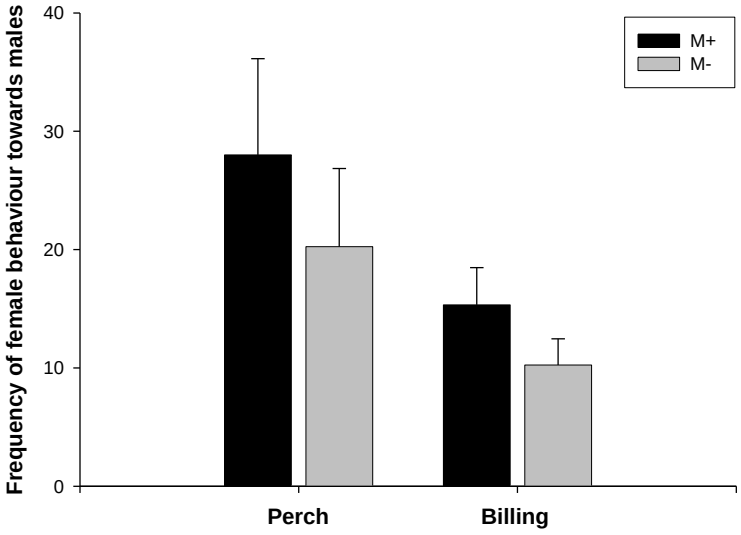


Figure 5. Mean frequency of female perch and billing towards more colourful M + and less colourful M - . The results are described as mean ± SEM. N = 12.

There were no differences between more and less colourful males in relation to their behaviour (Table II).

Table II. Comparison between males behaviour in response to female presence, by t-test for paired samples (M+ - more colourful male, M- - less colourful male, resp – time in the response area, perch – frequency of visits to the perch, bill – frequency of billings, shock – frequency of shocks in the glass. t – value of the test, p – value of the probability, N=12).

	M+		M -			
	Mean	Std error mean	Mean	Std error mean	t	p
resp	1503.55	93.76	1536.47	78.94	-0.496	0.630
perch	39.42	7.41	48.83	10.50	-0.888	0.394
bill	34.92	6.84	24.50	6.09	1.723	0.113
shock	1.83	0.79	4.25	1.29	-1.912	0.082

Experiment II

Female showed no preference, since there were no differences between female's behaviour towards males (Table III, IV and Figure 6 and 7).

Table III. Comparison between female behaviour in response to males in Experiment II, by t-test for paired samples (F – female, M+ UV - more colourful male submitted to UV treatment, M- ctrl- less colourful male submitted to control treatment, resp – time in the response area, perch – frequency of visits to the perch, bill – frequency of billings. Test: t – value of the test, p – value of the probability, N=12).

	M+ UV		M- ctrl			
	Mean	Std error mean	Mean	Std error mean	t	p
F resp	904.25	129.27	714.95	96.46	0.885	0.395
F perch	22.25	8.60	21.00	8.22	0.376	0.714
F bill	18.83	3.93	17.00	4.81	0.472	0.646

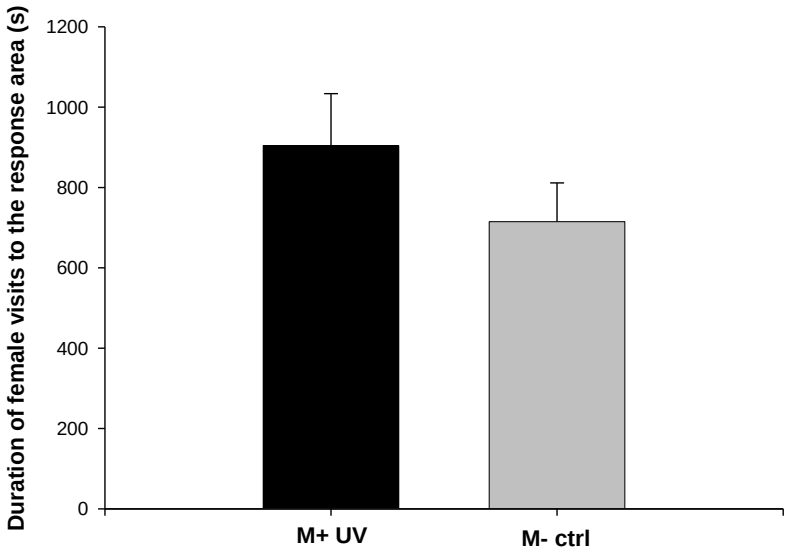


Figure 6. Duration of female visits to the response area (seconds) of more colourful males with UV treatment (M+ UV) and less colourful males with control treatment (M- ctrl), in the Experiment II. The results are described as mean ± SEM. N = 12.

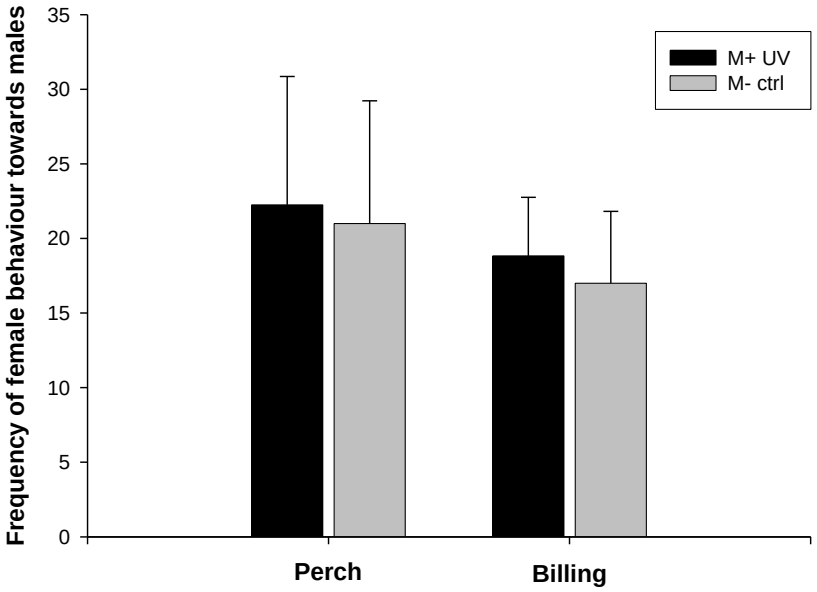


Figure 7. Mean frequency of female perch and billing towards each male, more colourful with UV treatment (M+ UV) and less colourful with control treatment (M- ctrl). The results are described as mean ± SEM. N = 12.

In this experiment, less colourful control males made significantly more visits to the perch ($p = 0.042$) and showed a higher frequency of billing, although the last one was not marginally significant (Table IV).

Table IV. Comparison between males behaviour in response to female presence, by t-test for paired samples (M+ UV - more colourful male submitted to UV treatment, M- ctrl- less colourful male submitted to control treatment, resp – time in the response area, perch – frequency of visits to the perch, bill – frequency of billings. Test: t – value of the test, p – value of the probability, N=12, asterisk indicate statistical significance).

	M+ UV		M - ctrl			
	Mean	Std error mean	Mean	Std error mean	t	p
resp	1702.65	50.41	1678.64	52.87	0.273	0.790
perch	26.25	7.73	69.25	23.84	-2.294	0.042 *
bill	55.17	8.38	84.50	15.49	-2.189	0.051

Experimental effect in the individuals

To understand if there was a possible effect in female's behaviour and motivation between the two consecutive trials, we compared the total behaviours performed by females in the first experiment with the second experiment. The consecutive experiments did not show any effect in females as they showed similar activities in both trials (Table VII)

Table V. Comparison between the Experiment I and II of the total behaviours performed by females to both males, by t-test for paired samples (resp total – total time in the response area for both males, perch total – total frequency of visits to the perch in both males, bill total – total frequency of billings to both males. Test: t – value of the test, p – value of the probability, N=12).

	Experiment I		Experiment II			
	Mean	Std. Error Mean	Mean	Std. Error Mean	t	p
Resp total	1700.25	19.74	1619.21	78.95	0.911	0.382
Perch total	48.25	14.37	43.25	16.49	0.352	0.731
Bill total	25.58	4.37	35.83	7.88	1.439	0.178

To check for any possible effect of the treatment (UV and Control chemical applied in both males), in male's behaviour and motivation we compared the behaviours performed by males in the first and the second experiment.

Table VI. Comparison between the Experiment I and II of the total behaviours performed by females to both males, by t-test for paired samples (M+ - more colourful male, M- - less colourful male, resp – time in the response area, perch – frequency of visits to the perch, bill – frequency of billings. Test: t – value of the test, p – value of the probability, N=12, asterisk indicate statistical significance).

		Experiment I		Experiment II			
		Mean	Std. Error Mean	Mean	Std. Error Mean	t	p
Resp	M+	1503.55	93.76	1702.65	50.41	-1.706	0.116
	M -	1536.47	78.94	1678.64	52.87	-2.135	0.056
Perch	M+	39.42	7.41	26.25	7.73	1.136	0.280
	M-	48.83	10.50	69.25	23.84	-0.867	0.404
Bill	M+	34.92	6.84	55.17	8.38	-2.902	0.014 *
	M-	24.50	6.09	84.50	15.49	-4.126	0.002 **

The experiments didn't show effect in most of males' behaviours, as they showed similar activities in both experiments, except for billing that increased in both groups of males in the Experiment II.

Morphometry, Colouration and Parasites

To check for any possible difference between more colourful and less colourful males, we compared morphometric parameters and the parasites between the two types of male. There was only a statistical difference for the length of the beak with the more colourful males having longer beaks. Beaks of these males also tended to be larger.

Table VII. Comparison of morphometric parameters and parasites between more coloured (M+) and less coloured (M-) males, by one way ANOVA. (F – value of the test, p – value of the probability, N of M+ = 8, N of M-= 9, asterisk indicate significance)

		N	Mean	Std. Error	F	p
Weight	M +	8	11.03	0.19	0.138	0.716
	M -	9	10.92	0.24		
Right Wing	M +	8	71.50	0.66	0.004	0.952
	M -	9	71.56	0.63		
Tail	M +	8	49.31	0.74	1.766	0.204
	M -	9	48.21	0.42		
Tarsus	M +	8	14.71	0.22	0.035	0.855
	M -	9	14.77	0.24		
Beak height	M +	8	6.03	0.12	1.659	0.217
	M -	9	6.24	0.11		
Beak length	M +	8	7.37	0.08	8.302	0.011 *
	M -	9	6.99	0.09		
Beak width	M +	8	6.63	0.16	3.308	0.089
	M -	9	6.33	0.06		
Parasites	M +	8	4.38	0.99	0.019	0.892
	M -	9	4.22	0.55		

Colouration of the males, morphometric parameters and parasites were not related, except for the variable beak length that was related with colouration.

Table VIII. Parametric correlation of Pearson between the morphometric parameters and parasites with the colouration score. (p – value of the probability, N = 17, asterisk indicate statistical significance)

Colouration score		
	Coefficient of Correlation	**p**
Weight	0.235	0.365
Right Wing	-0.342	0.179
Tail	-0.109	0.678
Tarsus	0.187	0.472
Beak height	-0.186	0.475
Beak length	0.699	0.002 **
Beak width	0.041	0.877
Parasites	-0.080	0.761

Discussion

The influence of Carotenoid colouration in mate choice

Our results confirm a previous work (Monteiro, 2008) that female serins displayed a sexual preference for males with more yellow colouration. The plumage of the species studied, results from pigmentation by carotenoids (Stradi, et al., 1995), therefore females preferred males that have more carotenoids in their plumage. We didn't found any evident association in female preference for other variables, so it seems that carotenoid pigmentation of males is the main factor of preference.

The time spent in close proximity to each male was considered a variable of preference, supported by other studies (Amundsen, et al., 1997; Hill, 2006). Evidences from this species sustain the association time as a predictor of female preference. Female and male serins were submitted to the tests in their natural reproductive period (Mota, 1995). In the aviary it was noticeable behaviours typical of breeding period (Leitão, Pers. Obs.), since

males sang frequently and females, that are sensible to the male's songs, tried to build nests in their cages (Mota, 1999; Mota and Depraz, 2004). Also, some works have demonstrated that time in association is correlated with breeding success in the wild (Burley, et al., 1994; Swaddle and Cuthill, 1994).

The expression of carotenoid pigmentation has been proposed to reflect a honest signal of male quality that females use in mate choice (Hill, 1999). The carotenoid deposition can be influenced by the access to pigments, nutritional condition and parasites infection. In this work, we didn't find any evidence for the influence of ectoparasites in colouration, although in the serin it has been already demonstrated that ectoparasites have a negative impact in the plumage during moult (Figuerola, et al., 2003). Vicente (2006) have shown also that endoparasites didn't influence serins' colour. The effectiveness of parasites in ornamental plumages can possibly be explained by correlation of endo and ectoparasites (Hill, 1999). Another potential ways to understand the value of colouration can be through diet manipulation or even by immunity challenges.

The influence of UV in mate choice

The results of this study provide us evidences that UV-reflecting plumage in the serin is an important component of the visual information, as females didn't exhibit any preference after reducing the UV of previous preferred males. This result is supported by other studies that demonstrated the function of UV-wavelength in mate choice, where females avoid males whose UV colouration was reduced (Maier, 1993; Bennett, et al., 1996; Andersson and Amundsen, 1997; Hunt, et al., 1997). Also in the serin, Monteiro (2008) has found similar results when performing experiments with filters.

Comparison between UV filters and UV chemicals

The current study and Monteiro's work (2008) present similar results but with different techniques applied. In this work we applied a more selective technique that only affects the colour of the male, keeping the background normal, and not disturbing the perception of the male regarding female. When using filters, the UV is blocked not only in bird colouration but also the background, possibly decreasing the male contrast with the

background or even creating an unnatural environment. By applying the chemical directly in the male, we can understand more clearly if the effect of the manipulation for females is due to male's colouration. It's important to maintain the background as normal as possible, since plumage reflectance against a natural background increases UV conspicuousness (Andersson, et al., 1998), and carotenoid plumage may be selected through enhanced background matching (Delhey, et al., 2010).

Comparing both methods, it seems that the application of the UV-reducing chemical and the control only increases preening behaviour (in both types of males), not changing any other variables. Korsten and colleagues (2007) have shown in blue tits that this method didn't affect couples that were already matched, or affected divorce, as well as not showing any side effects. The application of UV-absorbing chemical was effective in reducing UV reflectance and duck preen gland oil showed similar spectrum of natural condition.

The Meaning of UV reflection

It was hypothesised that UV may have a special role in communication among birds (Guilford and Harvey, 1998). Indeed, UV signals may serve birds in many functions and benefits (Cuthill, et al., 2000b), like short-range signalling and its imperceptibility for mammal predators, and may be effective as indicators of quality or even amplify a behaviour signal (Hausmann, et al., 2003). But, although some works indicate evidences for the importance of UV signals in mate choice (Bennett, et al., 1996; Cuthill, et al., 1999; Siitari, et al., 2002), most of the works didn't investigate methodically the importance of different parts of the avian visual spectrum (Stevens and Cuthill, 2007).

Hunt and colleagues (2001) tested for the first time, with zebra finches, whether the UV is a special waveband in the mate choice, by removing different parts of the spectrum. Their results suggest that females prefer males that show longer wavelengths, and so, didn't find any evidence for special role of UV.

The absence of preference after the application of a UV-absorbing chemical raises the question about the relative importance of different parts of the spectrum for the Serin. Females can be either attracted by UV, or perceive the male's ornamentation as Yellow+UV.

Bennett (1997) demonstrated in starlings, that UV was not correlated with variations in the visible spectrum. This was also demonstrated in the serin (Monteiro, 2008), where significant differences in the visible colouration of plumage among males were not seen in the UV. If the UV spectrum was more relevant, females could choose the less coloured males as they presented the same variation in UV. A full spectrum is probably essential for normal colour perception (Hunt, et al., 2001), so the yellow colouration in serins most likely corresponds to a combination of yellow and UV, and in the absence of the UV, yellow is perceived as another colour.

Even though bird's UV wavelength plays part in colouration, as proved by the fact that serins' plumage is affected by the removal of UV wavelength, the high UV reflectance plumage, like in blue tit (Hunt, et al., 2001), is more suggestive of signalling functions. Still, when a UV effect is found, one should be careful to consider that more important than other parts of the spectrum (Banks, 2001). It would be worth investigating whether in this species, UV works exclusively as a special channel or just as part of the overall colouration in mate choice.

In this work, we confirmed that the yellow carotenoid plumage colouration is sexually selected, as females are more responsive to this. We also demonstrated that UV wavelength is a component that influences mate choice, controlling for background. However, the yellow colouration in Serins seems to correspond to a combination of yellow and UV (Monteiro, 2008), and in the absence of UV, yellow is perceived as another colour. Further work investigating the different parts of the spectrum (UV, SW, MW and LW), is needed to confirm the relative influence of each part in visual signals.

Chapter 3 Influence of plumage maintenance behaviour in Serin's mating behaviour

Introduction

One of the most common signs of sexual selection in birds is the elaborate colourful plumage ornaments (Andersson, 1994). In several species, ornamental traits are signals of individual quality since there are physiological costs, vulnerability to predators and time and energy consuming to maintain (Walther and Clayton, 2005; Delhey, et al., 2007).

Maintenance of plumage have several functions, like cleaning, feather care (Clayton, 1991; Cotgreave and Clayton, 1994) and removal of ectoparasites and other microorganisms (Kulkarni and Heeb, 2007; Møller, et al., 2010). Thus, maintenance behaviour may have a signalling effect to mate choice, since females can benefit by choosing a mate based on honest information (Zampiga, et al., 2004; Amat, et al., 2010).

With so many functions, it's not surprising that maintenance behaviour occupies some amount of day time. Birds in wild spend 9.2 % of the day in maintenance behaviours, where males devote more time to maintenance of the plumage than females, being grooming (preening and scratching) the main activity (92.6%) (Cotgreave and Clayton, 1994).

Preening can be defined as " the bird pulling its feathers between the two mandibles of the bill, or nibbling the feathers with the bill tips" (Clayton, et al., 2010). Preening is associated with the production of secretions by the bird itself, being the uropygial gland an essential part of this behaviour. The uropygial gland exists in most of bird species and is located in the posterior part of the body at the beginning of the tail. The secretion produced by this gland consists in a complex mixture of waxes, sterols and hydrocarbons that help preserving the feather structure, confer more impermeability (Jacob and Ziswiler, 1982), delay feather wear (Moreno-Rueda, 2011), defends feather against microorganisms (Kulkarni and Heeb, 2007; Møller, et al., 2010), can help in odour communication (Jacob, et al., 1979) or even enhance colouration (Piersma, et al., 1999; Amat, et al., 2010).

The deliberate application of substances in the plumage, such as the preen oil, may modify plumage colour (Delhey, et al., 2007; Amat, et al., 2010) and signal a status or a condition to mate selection. Birds may renew colour and feather after moult, since there are several factors that affect the expression of ornamentation and therefore affect the signalling (López-Rull, et al., 2010).

The application of substances that modify the colour (visible and UV), also called cosmetic colouration, have been recognized as playing an important function in the maintenance of the feathers (Delhey, et al., 2007; Amat, et al., 2010; López-Rull, et al., 2010).

In addition to the preening behaviour and the possible involvement of uropygial oil, the morphology of the bird can influence the plumage maintenance. Clayton and Walther (2001) suggest that selection for efficient preening can play a role in the evolution of bill morphology, as they found that in 52 species of birds belonging to 13 families, birds with longer overhang bill had fewer lice.

We propose to clarify the importance of maintenance behaviour for mate choice in the Serin, *Serinus Serinus*.

Our main goals are to verify if females consider preening as quality indicator or in contrary reject it as a negative signal related to ectoparasites infestation.

Material & Methods

This experiment was made with different populations of other chapters, captured in 2008 and 2010 near Coimbra, Portugal, between January and March. The capturing, housing, identification, morphometry, parasites counting, and colour extent measurement of the birds are identical to the procedure described in Chapter II (data in Appendix III – A).

Colouration was measured before the experiments, in four coloured areas – crown, bib, breast, yield, with the same material and procedure as described in Chapter II. Then we averaged each colour variable for each zone, and calculated the variables according to Montgomerie (2006) (formulas presented in Appendix I - C): full brightness, brightness UV, hue, saturation and saturation total UV.

We calculated a general colour score based on a Principal Component Analysis (PCA), with the colour variables obtained by spectrophotometry (Appendix III - B). The first factor of the PCA explained 27.414% of the variation in colour among males, discriminating saturation and brightness, both variables negatively correlated (Appendix III

- C). We ranked males considering saturation, being the most colourful males the ones with highly saturated ornamental plumage colouration. We then separated males in groups of more colourful (M+) and less colourful (M-) and chose one from each group to form pairs to mate choice tests.

The mate choice trials were carried out from March to June, 2008 and 2010. We used the same procedure as described in Chapter II. Briefly, the experiments were performed in a three-compartment indoor aviary (Appendix I - D), in the morning, being in the first 30 minutes to familiarize to the new environment, and the trials lasted for 30 minutes In each compartment, the closest area of female-male association was designated as response areas, where it was register time spent, and preening behaviour of both males and female (complete Ethogram in Appendix I - A).

Each trial had a new female (N=22) and a total of 34 males were used as stimulus. The trials consisted in a unique combination of male pairs, being one more colourful and the other less colourful.

The tests were considered to the analysis if during the period of habituation female following the validation criteria described in Chapter II. The tests were recorded using a video camera Sony SSC-DC378P to a VCR.

The behaviour of the individuals was analysed using the software Observer ® 9 (Noldus Information Technology). From the second half hour of the test it was measured the following variables in both sexes: "Response Area" (Duration of visits to response area), "Preening" (Duration of preening behaviour); Only in males we measured: "Preening Female" (Duration of preening when female was in front of male), "Preening No subject" (Duration of preening when female was not in front of male).

Statistical analysis

The statistical analysis was performed using software IBM SPSS Statistics ® 19.0 for Windows. We analysed the mate choice in females, females and male's preening, the differences in male's characteristics and the correlation between preening behaviour and any male characteristic. To check the distribution of the data we tested the variables by One-sample Kolmogorov-Smirnov test, and when data were normally distributed we used parametric statistics in the analysis, when not, even after transformation, we used non parametric statistics.

For mate choice, we conducted a paired sample t-test to check for any difference in female's behaviour towards each male. For male's preening behaviour, we made a paired sample t-test for the total time of preening. To check for differences in male's preening behaviour was due to female's presence, we check whether there were differences in each male's preening behaviour with and without the female's presence by Wilcoxon two related-samples test.

To analyse a possible difference between more colourful and less colourful males on a morphometry parameter and parasites, we performed a one-way ANOVA.

To check for any relationship between male's preening behaviour and colouration, morphometry and parasites, we performed Pearson bivariate correlations.

In all tests, the level of statistical significance was 0.05.**Results**

Female mate choice and preening behaviour

Females spent more time in the response area of M+ males (p=0.041, Table IX) then in the in area of M-, showing their preference for them. They also performed preening during more time in front of more colourful males (p=0.042) – Figure 8.

Table IX. Comparison between female behaviour in response to males, by t-test for paired samples (F – female, M+ - more colourful male, M- - less colourful male, resp – time (s) in the response area, pree – Proportion of time (%) of female preening behaviour relative to the time that they stayed in each male area. Test: t – value of the test, p – value of the probability, N=22, asterisk indicate significance).

	M+		M -			
	Mean	Std error mean	Mean	Std error mean	t	p
F resp (s)	1045.787	98.110	623.664	102.617	2.178	0.041 *
F pree (%)	12.276	3.275	4.486	1.496	3.057	0. 042 *

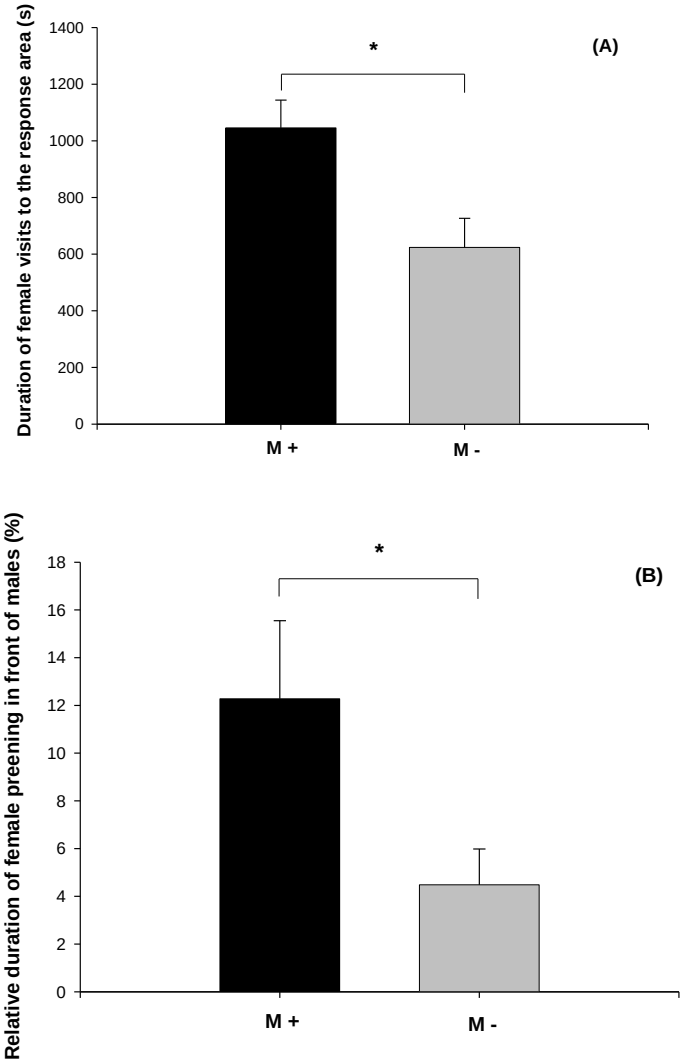


Figure 8. (A) Duration of female visits to the response area (seconds) and **(B)** Relative duration of female preening in front of males (%), of more colourful males (M+) and less colourful males (M-). The results are described as mean ± SEM. N = 22.

Male preening behaviour

In relation to male's behaviour, in the total time of the trial, more colourful males spent more time preening than less colourful males, being almost statistically significant (Figure 9 and Table X).

Table X. Comparison between more colourful and less colourful males and their preening behaviour, by t-test for paired samples (F – female, M+ - more colourful male, M- - less colourful male, Male Total Pree F – Total of male's preening behaviour (%), that includes in front and not in front of female; Male Pree F – Proportion of preening time (%) in front of the female, relative to the total time that female stayed in the specific response area. Test: t – test value; p – value of the probability, N=22).

	M+		M -			
	Mean	**Std error mean**	**Mean**	**Std error mean**	**T**	**p**
Male Total Pree F (%)	10.314	2.077	5.865	0.879	2.067	0.051
Male Pree F (%)	13.425	3.376	7.1878	3.113	1.261	0.221

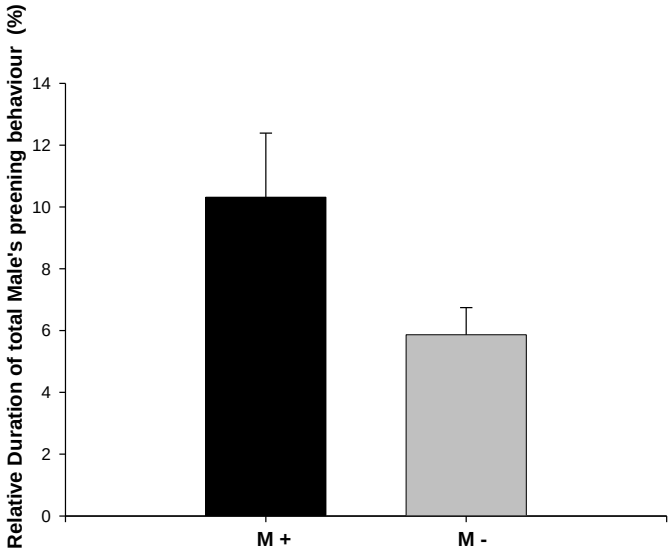


Figure 9. Relative duration of total male's preening behaviour (%) of more colourful males (M+) and less colourful males (M-). The results are described as mean ± SEM. N = 22.

To understand if the increased preening was due to female's presence, we checked whether there are differences in each male's preening behaviour with and without the female's presence. We didn't find any statistical differences in male's behaviour in female's presence or absence (Table XI, Figure 10).

Table XI. Comparison between male's behaviour on female's presence and absence, by Wilcoxon 2-related-samples test (F – female, M+ - more colourful male, M- - less colourful male, presence – time that female was in front of male, absence– time that female was out of the area or in the other male's area. Test: z – value of the test, p – value of the probability, N=22).

	F presence		F absence			
	Mean	**Std Deviation**	**Mean**	**Std Deviation**	**z**	**p**
M +	13.425	3.376	7.967	3.057	-1.232	0.218
M -	7.188	3.112	6.025	1.330	-0.161	0.872

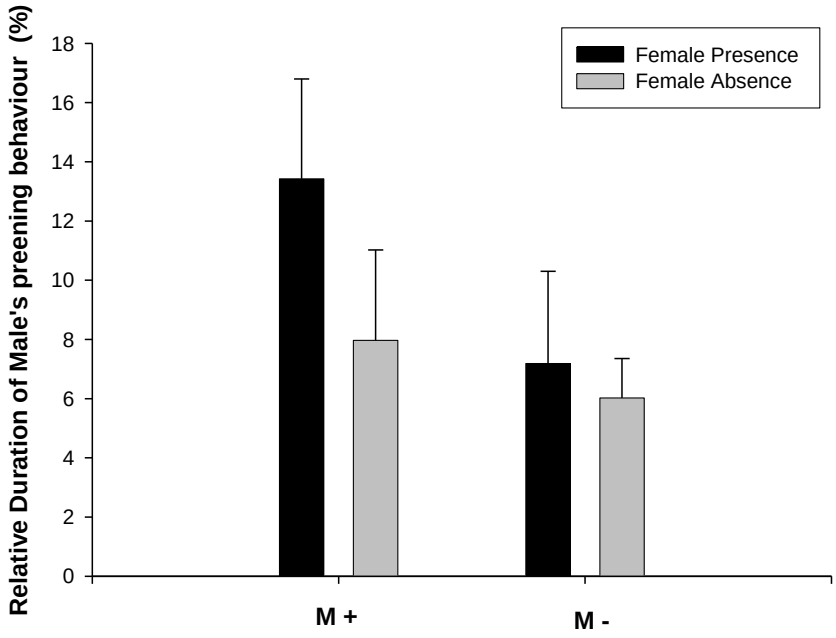


Figure 10. Relative duration of male's preening behaviour (%) of more colourful males (M+) and less colourful males (M-), in presence and absence of females. The results are described as mean ± SEM. N = 22.

Morphometry, Colouration and Parasites

We checked for a possible influence of morphometric parameters, colouration and parasites in preening behaviour. Time for preening behaviour in males was not related with any variable (Table XII).

Table XII. Parametric correlation of Pearson between the total preening behaviour and morphometric parameters, parasites, colour extension and colour score. (p – value of the probability, N = 34).

Total Preening Behaviour		
	Coefficient of Correlation	**P**
Weight	0.189	0.285
Right Wing	0.072	0.684
Tail	-0.036	0.840
Tarsus	-0.008	0.965
Beak height	0.064	0.721
Beak length	-0.151	0.395
Beak width	0.063	0.725
Parasites	-0.040	0.824
Crown Extension	0.092	0.607
Breast Extension	0.106	0.552
Colouration Score	-0.238	0.176

Discussion

In the present study, females preferred more colourful males, as described in previous studies (Monteiro, 2008; see Chapter II for results) and they also performed preening during more time in front of these males. This may be suggestive of a possible role of preening as a courtship behaviour in serins, similar to pigeons (Fabricius and Jansson, 1963) that shows a strong association in the sequence of behaviours preening followed by copulation. Courtship behaviours are good indicators of a female tendency to mate with a particular male (Jennions and Petrie, 1997).

Moreover, our results showed that more colourful males spent more time in maintenance behaviour than the less colourful ones (average: M+ 13.425%, M- 7.967%). The difference was only marginal to significance mostly due to a greater variance in more colourful males. A larger sample size should have been used in order to have a clear measured effect. To our knowledge, this is the first experiment that provides evidence for differences in preening time dedication depending on individual colouration. Other works have shown that individuals with longer plumages devoted significantly more times do maintenance behaviour that the ones with shorter plumage (Walther and Clayton, 2005) and also, individuals that were dirtied experimentally devoted more time to preening (Zampiga, et al., 2004; Griggio and Hoi, 2006; Griggio, et al., 2010a).

When comparing male's preening behaviour in presence and absence of female, we didn't find any differences, as males preened even when females were not in face of them. Similar results were described by Griggio and Hoi (2006) and Zampinga (2004) with budgerigars, suggesting that males don't perform preening to display towards females. In addition, increased preening behaviour was not related to ectoparasites number, or any morphometric parameters. These results don't support the hypothesis of a possible relation between preening behaviour and ectoparasites number. Nevertheless, we don't rule out the possibility that individuals with high number of ectoparasites increase their preening behaviour, although in this case the effect is not seen, probably because these individuals present an overall low number of ectoparasites.

Overall, our findings indicate that female mate choice can be based not only in the acquired colouration by individuals during moult, which is usually obtained long before the mate choice period, but also in the amount of time and energy that males dedicate to maintain plumage in a good condition. Preening behaviour may influence the plumage reflectance spectrum (Zampiga, et al., 2004), specifically structural colours that affect UV wavelength (Griggio, et al., 2010a) or it can have a signaller effect on which females base their choice (Griggio and Hoi, 2006).

Moreover, these results open the possibility for a role of preening in cosmetic colouration in serins. Lopez et al. (2010) have shown that individuals that were experimentally prevented from using uropygial secretions in preening, increased brightness

and decreased Yellow and Red saturation. Likewise when they applied preen oil deliberately in museum specimens they observed an increase of yellow and red saturation and a decrease of brightness. In flamingos, *Phoenicopterus roseus,* Amat et al. (2010) have shown that individuals that performed more application of the uropygial oil enhanced more their colour and initiated nesting earlier. If serins are capable of enhancing their colouration through uropygial oil, preening has a more important role than we actually know.

In conclusion, it's clear that maintenance behaviour is an essential element of avian behaviour (Cotgreave and Clayton, 1994), and our results suggest that it may also be important to female mate choice. Further work is necessary to understand the relative importance of preening behaviour in mate choice by comparing the behaviour performance outside the breeding season. Furthermore, it's important to investigate if uropygial oils are involved in maintenance behaviour and have a function in cosmetic colouration in this species.

Chapter 4 Personality and Mate Choice in the Serin

Introduction

In many animal species, individuals exhibit consistent individual differences in their behaviour (Sih, et al., 2004), such as in aggressiveness, activity, exploration, risk-taking, fearfulness (Réale, et al., 2007) among others. Although these behavioural traits can be circumstance susceptible, some individuals are consistently more exploratory, aggressive or active than others. This individual stability of correlated behavioural traits, is often defined as personality traits (Gosling, 2001; Réale, et al., 2010a), behavioural syndromes (Garamszegi, et al., 2009), temperaments (Réale, et al., 2007) and copying styles (Coppens, et al., 2010). Animal personality refers, in a broad definition (Réale, et al., 2007), to behavioural differences among individuals, that can be broaden to the family level, population or species, and that are consistent over time and in different contexts.

Inter-individual variation of behaviour can be differentiated on several dimensions (or personality phenotypes) commonly distributed along a continuum (Gosling 2001), like 'shyness–boldness', 'exploration-avoidance, ''proactive-reactive', or 'passive-aggressive' and (Réale, et al., 2007), 'fast-slow' (Verbeek, et al., 1994; Carere, et al., 2005). On the extremes of the continuum, individuals can be considered as having different behavioural strategies. On one end of the continuum are "bold", "fast", "proactive" individuals, which are more active, aggressive, and faster in exploring new environments and foraging. At the other extreme are "shy", "slow", "reactive" individuals, which are more caution in their decision, neophobic, less active, aggressive and explorative.

The evolutionary mechanisms that maintain these variation "of extremes" between populations may follow the trade-off hypothesis in which the individual fitness varies depending on context.

Although the upsurge of studies in the last decade (Réale, et al., 2010b), the evolutionary origin of personality and its maintenance is still unknown. While various hypotheses have been proposed, sexual selection has been scarcely referred, but it is possible that it may play an important role in the origin and maintenance of personality traits (Schuett, et al., 2010). A few works tried to correlate sexual selection with personality traits. Godin and Dugatkin (1996) were the first to show a direct preference for a personality trait in guppies, where females prefer bolder males, independently of their colouration. Other studies with zebra finches (Forstmeier and Birkhead, 2004; Schuett, et

al., 2011) and with great tits (Groothuis and Carere, 2005), showed individual differences in preference for personality of sexual mates.

In this study we subjected male and female serins to several contexts to measure behavioural responses that could be used to access proximate level explanations to personality and relate it with mate choice. Our objectives are i) to study individual variability in behaviour across contexts in the serin, ii) understand how sexes differ in their behavioural traits and ii) to explore a possible correlation between mate choice and behavioural traits in other contexts.

Material & Methods

We carried out the experiments between January and June 2011, in the Laboratory of Ethology, University of Coimbra, Portugal. The capturing, housing, identification, morphometry, parasites counting and colouration assessment of the birds are identical to Chapter II, as this experiment was run at the same time with the same birds. All the data can be found in Appendix II.

Behavioural tests

Adult serins (16 males and 16 females), were subjected to several behavioural tests as described below. The aim of the present study was to relate the results of behavioural tests with personality traits, as illustrated in the framework in Figure 11. All tests were carried in a separate room adjacent to the aviary, except for the new object. The tests were assessed in the morning period to avoid behavioural changes due to day time. Individuals were tested in random order.

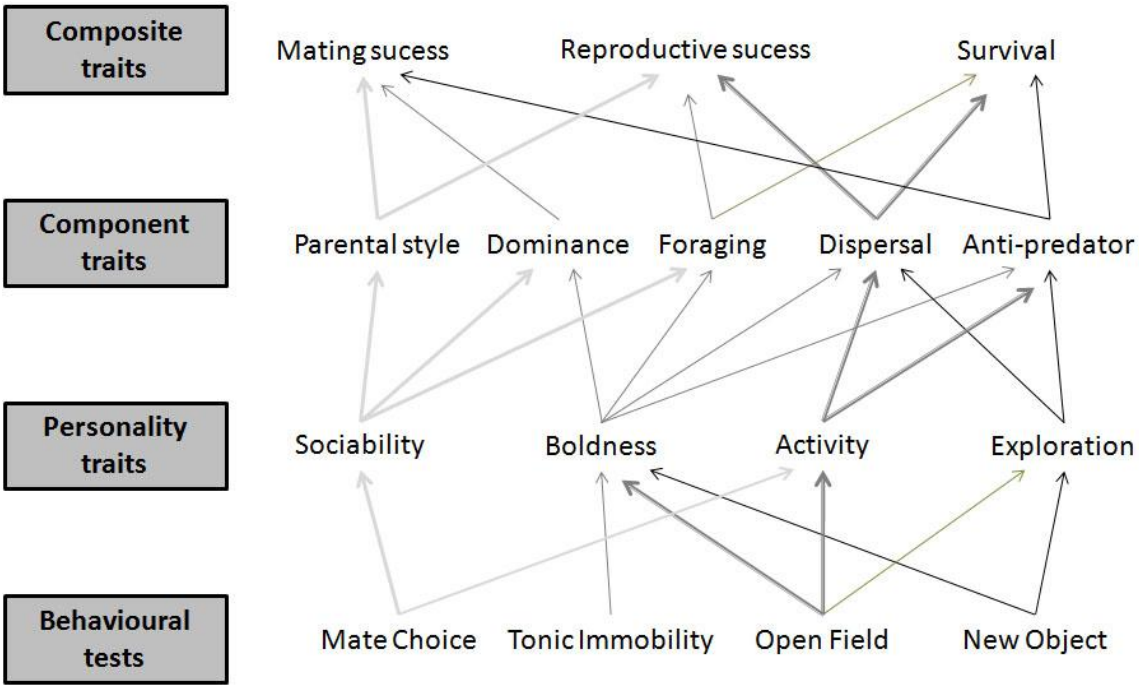


Figure 11. Flow diagram illustrating the framework of the present study for personality traits. Adapted from Réale and colleagues (2007).

Tonic immobility

Tonic immobility (TI) test assesses latency to pass from a motor inhibition resulting from a stressful event, to a mobile state (Gallup, 1974). For this purpose, birds were removed from their home cage and hold with belly up, covered for dark, for one minute and carried to an adjacent room. After it, they were placed in a support near the experimenter (Figure 12) and duration of TI was recorded for one minute. Birds that did not fly away were given a maximum score of 60 seconds. Birds that react immediately when turned on their back and flied were considered non-TI individuals, and others that stayed were considered as TI individuals.

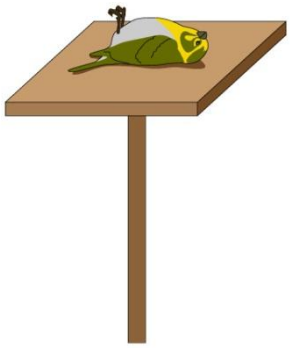


Figure 12. Illustration of tonic immobility test. Individuals were placed with belly up in a support (1 meter high) with the observer half meter distant.

New Object

Neophobia was assessed similar to other studies (Carere, et al., 2005; David, et al., 2011), that focused on phobia and exploration behaviour. In the individual's home cage, with 4 perches of regular intervals (Figure 13), food was removed 1 to 2 hours before the test. After it, all the home cage individuals, except for the focal individual, were removed for the trial. By inserting a partition to separate the individual from the object, we introduced an unfamiliar object – a yellow clothes peg - in the perch more distant to the individual with a food recipient next to it. The partition was removed and the individual was recorded for 10 minutes (600 seconds). We changed the object and feeder's position, for each test, alternating the sides of the cage to control for any possible side effect.

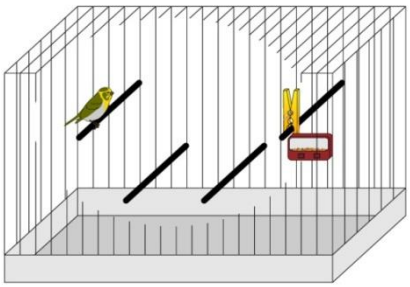


Figure 13. Illustration of new object test. On the left side the focal subject, on the right side the new object (clothes peg) and the feeder.

Behaviour analysis took into account the activity between perches, the latency to perch near the object and to the feeder. None of the birds pecked the object. Birds that did not approach object and feeder were given a maximum score of 600 seconds. Although the test was made in a known environment (home cage), the variable "activity between perches" was considered exploration, since the individual was exposed to a new context – with a new object, without the normal quantity of food and the other subjects of the home cage.

Exploratory Activity

Exploratory activity was analysed in a novel environment as described by Verbeek (Verbeek, et al., 1994). The tests ran in an unfamiliar cage, containing five trees (four on each corner and one in the centre), each with six perches (Figure 14). At the beginning of each trial, the focal bird was introduced in a small opaque box inside the cage, for 1 minute, the box was opened at distance and individual exploratory behaviour was recorded for 10 minutes. Behaviour analysis was intended to account flights between trees and hops within trees for a score. However, serins did not respond to this test since all the individuals, when freed from the box, stayed the whole trial in one tree, without exploring. Thus, this test was excluded for further analysis.

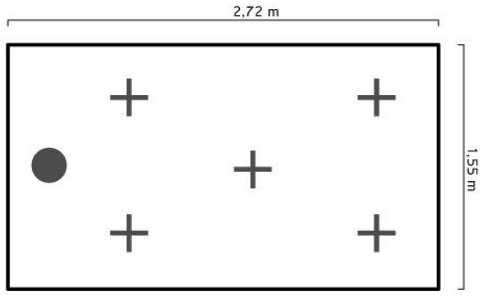


Figure 14. Illustration of exploration test. Birds were kept in the opaque box (represented by the circle), and after one minute, the box was opened at distance. It was recorded, for 10 minutes, the exploration of the bird through the 5 trees (represented by cross).

Mate Choice

Mate choice was assessed simultaneously to the work described in Chapter II, experiment I, as we used the same experiment for the present study, although we used 16 mate choice tests.

As described in Chapter II, the tests were ran in a three-compartment indoor aviary (Appendix I - D). Each test lasted for one hour, with on female and two males. Males were combined in more colourful and less colourful (based on the PCA: Appendix II - C) and presented to a random but different female. Males that were used more than one time were matched in unique pairs.

For the purpose of this study, in the first 30 minutes (1800 seconds), we wanted to measure latency for female to explore the response areas associated to each stimulus bird. Due to some problems related with the apparatus, we couldn't take this variable.

In the following 30 minutes, we measured behaviours of interest – billing, hopping, shock – and time spent in the response areas, as described in Chapter II (complete ethogram in Appendix I - A).

Behaviour analysis

Table XIII describes the variables taken for each personality trait. All the tests, except for tonic immobility, were recorded using a video camera Sony SSC-DC378P and a Canon HD Legria HFM306. The behaviour of the individuals were scored afterwards, using a computer with the software Observer ® XT (Noldus Information Technology) with help of an X-keys Jog & Shuttle.

Table XIII. Correspondence of variables taken from each behavioural test to a personality trait. Value of the test indicates the type of result in relation to the behavioural test - (+) - higher the value, more exploratory is the individual; (-) lower the value, less TI and neophobic is the individual.

Personality trait	Variables taken	Value of the test	Behavioural Test
Exploration	Exploration of the New Object (proportion of frequency of hops with time spent in perches)	+	Novel Object
Boldness	TI duration (s) Latency to the object (s) Latency to the food (s)	- - -	Tonic Immobility Novel Object Novel Object

Statistical analysis

The statistical analysis was performed using software IBM SPSS Statistics ® 19.0 for Windows. All the tests the level of statistical significance was $p<0.05$.

Tests were made with N= 32 (females=16, males=16). Variables were checked for normality by One-sample Kolmogorov-Smirnov test, and statistical transformations were applied if necessary to meet the parametric criteria. Variables, even after transformation, were not normally distributed so we used non-parametric tests.

To check for consistency between different behavioural tests and within each category, we performed spearman bi-variate correlations, to visualize the strength and direction of the relationships between the traits.

To determine if behavioural traits differed in gender we performed a Kruskal-Wallis Test for all the variables of the behavioural tests.

In order to attempt distributing the birds under a personality axis, the behavioural variables were used in a Principal Component Analysis (PCA), to determine a score for subsequent analysis based on the first PC. We used the eigenvalues superior to 1 criteria to retain factors.

The possible link between personality and mate choice was verified with personality score (obtained in the PCA) and several behaviour variables taken from mate choice test, also by spearman bi-variate correlations and partial correlations.

The possible effect of morphometric parameters, colouration and parasites on personality score was analysed by Spearman bi-variate correlations.

Results

Relationship within and across personality traits

When analysing within-individual Boldness (as described in Table XIII up), significant positive correlations were found between Latency to object and to the feeder (r_S=0.608 p>0.001), TI and Latency to the object (r_S= 0.435 p=0.013), and TI and Latency to the food (r_S= 0.359 p=0.044), indicating that individuals more neophobic are also the ones who take more time to react in a stressful situation (Figure 15).

Relationship between personality categories demonstrates a pattern of association between Exploration (New Object-Exploration) and Boldness (New Object – Latency to food) (r_S=- 0.341 p=0.056).

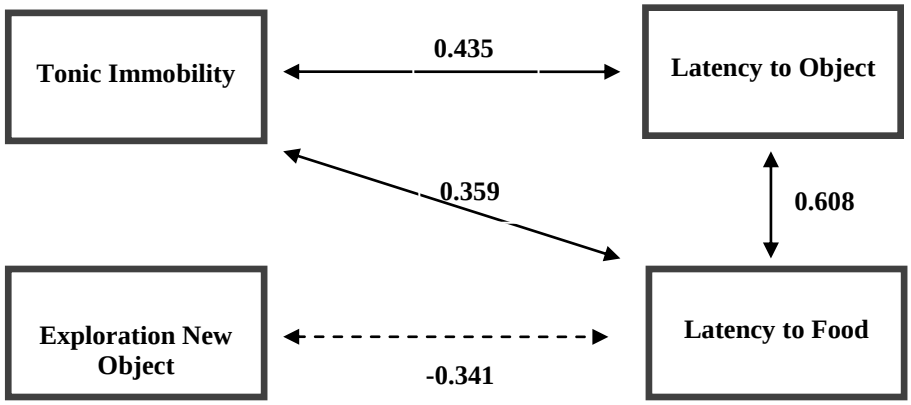


Figure 15. Relationship between behavioural tests. Black arrows indicate a significant correlation, whereas dashed arrow indicate marginally significant correlation (p=0.056). Spearman correlations are indicated in each line.

Differences between gender

To check for any possible difference between males and females in the results of behavioural tests, we compared both by Kruskal-Wallis Test. There were significant differences in TI, as females were more reactive than males. Also Latency to Food was nearly significant (p=0.051), with females taking more time to approach food than males (Table XIV).

Table XIV. Comparison of behavioural tests, between females (F) and males (M) by Kruskal-Wallis test. (Chi-square– value of the test, p – value of the probability, N of F = 16, N M-= 16, asterisk indicate significance)

Behavioural variable	Sex	N	Mean	Std. error	Chi-square	p
Tonic Immobility	F	16	41.025	6.572	9.787	0.002**
	M	16	15.499	5.768		
New Object - Activity	F	16	122.572	20.190	1.083	0.309
	M	16	117.816	35.033		
New Object - Latency to object	F	16	212.916	50.951	0.436	0.509
	M	16	206.826	60.767		
New Object - Latency to food	F	16	443.251	53.517	3.797	0.051
	M	16	276.970	62.312		

Scoring personality

We performed a Principal Component Analysis (PCA) with a set of variables (Table XIII) obtained from the behavioural tests, to classify individuals by their personality.

The PCA returned 2 significant factors, where the first factor (PC1) discriminated Boldness by negative values, explaining 43.51% of the variation, and the second factor (PC2) discriminated Exploration by positive values, explaining 28.263% (Appendix IV – A). Accordingly, PC1 discriminate by low values, individuals that are more active, exploratory and curious; in the opposite sense, high values indicate individuals that are less exploratory, less active and more neophobic (see Figure 16 for frequency distribution). The score retained from the first factor was used further to relate mate choice to personality and several parameters from the individuals (Appendix IV – Personality score).

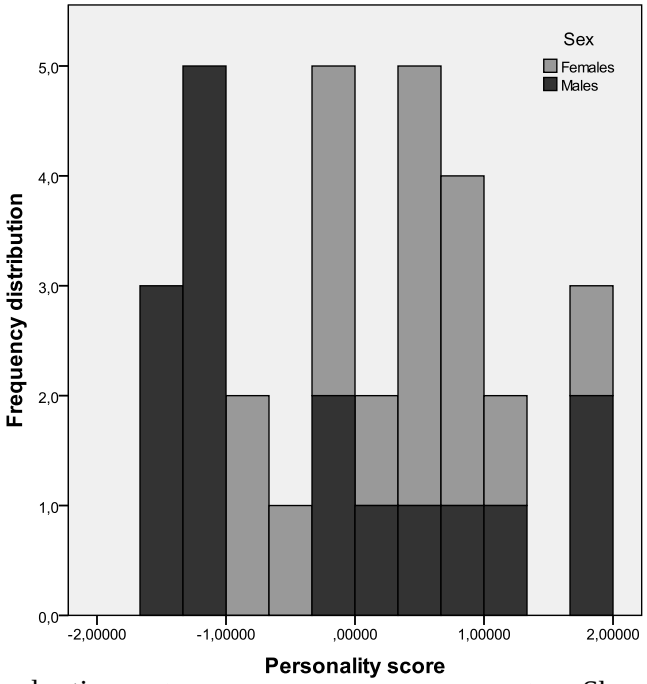


Figure 16. Individuals distribution in a personality axis. Low values of personality score indicate more explorative and risk-taking individuals. High values indicate neophobic and less active individuals

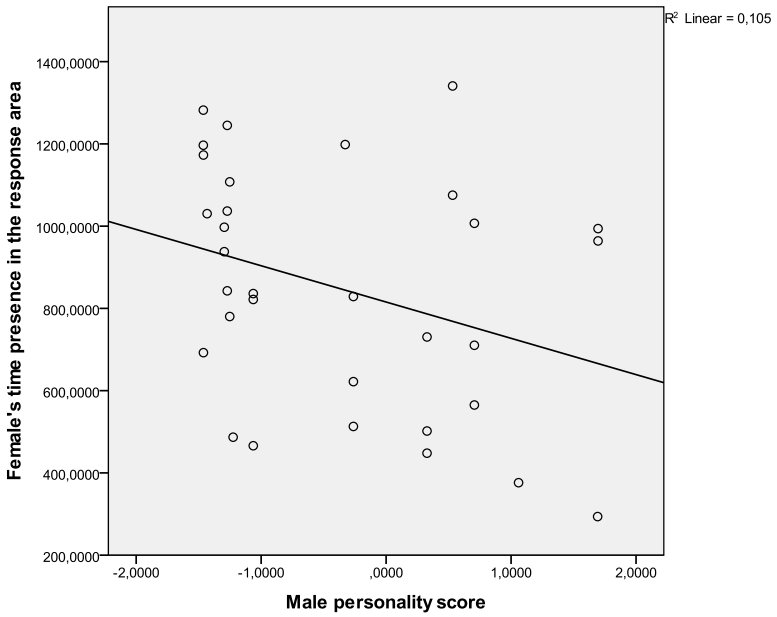
Influence of Personality in Mate Choice

Female

Females spent more time in the response area of more colourful males than the less colourful males (Chapter II for results). We checked for any correlation between female's behaviour during mate choice and male's personality score. Time spent in the response area of males was related to male's personality (r_S=-0.404, p=0.022) (Figure 17). All other variables were not correlated (Table XX).

Table XV. Spearman bi-variate correlations, between the Male's personality Score and time spent by females in the response area (interaction area with male), frequency of hops in the perches, frequency of billings and shocks (p – value of the probability, N = 16, asterisk indicate statistical significance)

Male's Personality Score		
	Coefficient of Correlation	**P**
Female's presence time in response area	-0.404	0.022 *
Female's frequency of hops in the perches	-0.036	0.844
Female's frequency of billings	-0.161	0.380
Female's number of shocks in the glass	0.066	0.718



Bold and active ◄·····► Shy and inactive

Figure 17. Influence of male's personality score on female's presence time in the response area of the respective male.

It was also tested if female's behaviour during mate choice was influenced by their own personality score. All variables were not correlated (Table XXI).

Table XVI. Spearman bi-variate correlations, between the Personality Score and time spent by females in the response area (interaction area with male), frequency of hops in the perches, frequency of billings and shocks (p – value of the probability, N = 16, asterisk indicate statistical significance)

Female's Personality Score		
	Coefficient of Correlation	**P**
Female's presence time in response area	-0.069	0.707
Female's frequency of hops in the perches	-0.157	0.390
Female's frequency of billings	-0.217	0.232
Female's number of shocks in the glass	-0.051	0.780

Male

We checked for any correlation between the male's behaviour during mate choice and their personality score, through partial correlations controlling for female's presence. Male's presence time in the response area is correlated with its own personality score (Figure 18). Frequency of shock is almost significant. All the other variables didn't show any correlation (Table XXII).

Table XVII. Partial correlations between the Personality Score and time spent by males in the response area (interaction area with female), frequency of hops in the perches, frequency of billings and shocks, controlling for female's presence (p – value of the probability, N = 16, asterisk indicate statistical significance)

Male's Personality Score		
Control: Female's presence	**Coefficient of Correlation**	**P**
Male's presence time in response area	- 0.484	0.006 *
Male's frequency of hops in the perches	-0.114	0.540
Male's frequency of billings	-0.076	0.686
Male's number of shocks in the glass	0.310	0.090

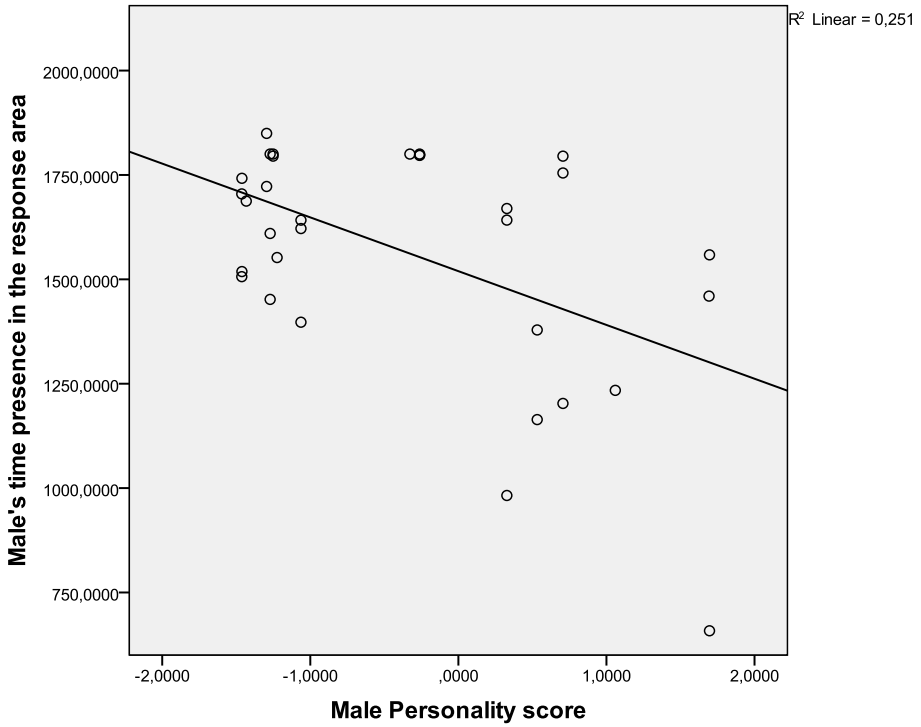


Figure 18. Influence of male personality score on male's presence time in the response area associated to the female.

Effect of morphometry, colouration and parasites

Individual variation in behaviour can depend in other factors that influence behaviour, like age and consequently experience, activity related with condition ability, etc. For this purpose, we checked for any correlation between personality score and age, morphometric parameters, parasites, and colouration (in males). We didn't find any variable related to personality scores (Appendix IV - C).

Discussion

Evidence for Personality traits

Our work provides information on the existence of behavioural differences related to personality in serins. We found correlations within Boldness behavioural tests, where individuals behaved consistently regarding their neophobic and tonic immobility responses.

In addition, the results suggest an association between two distinct personality traits, Boldness and Exploration (Réale, et al., 2007) which indicates a possible existence of a behavioural syndrome (Sih, et al., 2004).

PCA analysis discriminated Exploration and Boldness. By the factor score, we were able to characterize individuals in two dimensions related to their behaviour: bold-exploratory and shy-inactive. Birds who exhibited tonic immobility also had high latency to approach the object and the feeder, and also were less exploratory. In the other extreme, non-TI individuals were the ones that had low latency to approach the object and feeder and were more exploratory.

Ecological and evolutionary consequences of personality traits are expected to be significant because they have fitness repercussions (Sih, et al., 2004). Exploration and boldness probably play an important role in this species, on which depend food resources that are distributed in different types of environment and varies along the year (Newton, 1972; Mota, 1995). Generally, high exploratory individuals increase their risk of predation; still, this strategy also increases the chance of finding food resources.

Environmental pressures and population densities can affect competition for food or risk of predation (Elgar, 1989) and consequently maintain genetic variation in personalities (Dingemanse, et al., 2004). Also, the constitution of the group, where the individual is in, may also influence their behavioural strategy.

Behavioural variation between sexes

In the present work, females and males presented different results in some behavioural tests, and consequently, in their personality scores. In this population, females seem to be shyer and less active than males that exhibit a more proactive strategy. Few works had checked for personality differences in gender (van Oers, et al., 2008 with great tit; Schuett and Dall, 2009 with zebra finches), but all of them were successful in finding behavioural differences similar to the present work.

Sex differences suggest different selection pressures acting on male and female personality. This differences can be associated to distinct life history strategies (Senar and Domènech, 2011), different roles of the sexes in the reproduction (Schuett and Dall, 2009), different physiological reactions to stressful situations (van Oers, et al., 2008) and others. Further work is needed to understand better these differences.

Personality and Mate Choice

Mate choice has been recognized to be based not only in secondary sexual characteristics (Andersson, 1994), but also on behavioural traits (van Oers, et al., 2008; Schuett, et al., 2010).

In serins, as carotenoid based plumage is acquired by diet, more exploratory and bold individuals may have increased foraging abilities and consequently, consume more food rich in carotenoid. By preferentially mating with colourful males, females may be choosing relatively bold and high exploratory individuals, and so, this behavioural trait can be an additional indicative of male quality. However, in our work, we didn't find any relation between male's personality score and its colouration.

Despite that, we found a relation between time in association of females with bolder and more exploratory individuals, independently to the fact that they choose more colourful males. We also found out that bolder and more exploratory individuals stayed more time in the response area than shyer and inactive ones. This difference can be due to a difference in reproductive strategy. Generally, bold individuals in the wild or in captivity, have a higher reproductive success than shy, but in return have shorter lives. Thus, while shy individuals have a reduced reproductive success in a short term, they live longer and therefore may have identical overall fitness of the individuals bold (Smith and Blumstein, 2008).

Conclusion

Personality influences on ecology and evolution of species (Réale, et al., 2007) increases the importance of continued studies to better understand the role of personality variation in populations and between species. Here we found correlated personality traits

differences within females and males and between them. We also showed for the first time the effect of personality in the performance of the individuals during mate choice tests.

Further work is needed to check for consistency within behavioural traits in the serin and assess other personality traits related to other ecological contexts. Also it's important to understand the possible fitness consequences of personality traits in mate choice like quality, aggressiveness, foraging ability, antipredator responses, parental care, and others.

Chapter 5 General Discussion

In this thesis, we studied the serin, *Serinus serinus*, a monogamous finch that is sexual dimorphic (Newton, 1972). Within populations, males present variation in their carotenoid-based yellow plumage. The expression of carotenoid pigments has been proposed to be an honest signal of male quality as it is costly to produce (Hill, 1999). Mate choice experiments show that females display a preference based on the expression of this secondary sexual characteristic (Chapter 2 and 3).

Besides the human "visible" colouration, serins have UV vision like most passerine birds (Cuthill *et al.* 2000), and are thus able to perceive UV reflectance. We have demonstrated (Chapter 2) that, when using a selective technique to reduce UV wavelength directly in male's serin plumage, females didn't exhibit any preference, reinforcing that UV influences mate choice: either males lose their attractiveness in the absence of UV or, yellow colouration corresponds to a combination of yellow and UV, and in the absence of the UV, yellow is perceived as another colour.

It is not only the production of colouration that is expensive, it can be also costly to maintain, as it involves physiological constrains, increased risk of predation and time spent in cleaning and maintenance (Walther and Clayton, 2005). Plumage maintenance behaviour can be an important influencer in mate choice, either as a complement of signalling in courtship or be an important behaviour to high quality individuals. Here, we have studied the importance of preening behaviour (Chapter 3) in male performance and female preference. The results suggest that preening is carried out by individuals to reinforce their appearance, as more colourful males performed more preening than less colourful ones.

Behavioural strategies can also influence mate choice, by differences in the performance of the displays in courtship or even influence *a priori* condition state of the individuals. In Chapter 4, we described consistent differences in behaviour of serins in different contexts, referred as personality traits that have not previously been explored in this species. Individuals were discriminated in two dimensions related to their behaviour, bold-exploratory and shy-inactive. I also investigated for the first time in birds the effect of personality in the performance of the individuals in mate choice tests. We found differences in behavioural responses, as bolder and exploratory individuals were also more active during the trials in detriment of shy and inactive ones.

This thesis provides a broad view of how different factors such as colouration, plumage maintenance behaviour and personality traits can influence the behaviour ecology of the serin, specifically the mate choice in this species. Taken together, we expect that these results motivate future work to better explain ecological and evolutionary significance of sexual selection in serins, especially focusing on (1) investigating the different parts of the colouration spectrum (UV, SW, MW and LW), to confirm the relative influence of each part in visual signals; (2) explore the hypothesis of cosmetic colouration through uropygial oils in this species; (3) check for consistency within behavioural traits in the serin and assess other personality traits related to other ecological contexts to understand the possible fitness consequences of personality traits in mate choice.

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Appendix

Appendix I - Material and Methods

(A) Complete Ethogram

Title	Code	Subject	Description
Actions in Mate choice			
Duration in Response area	resp	Female and Male	Time period that the individuals are in the response areas, including the perches.
Hopping in perches	perch	Female and Male	Frequency of hops in the perches
Billing	bill	Female and Male	Frequency of billing (touching with the beak in the perch)
Shocks	shock	Female and Male	Frequency of shocks in the glass that separate the compartments.
Preening	pree	Female and Male	Time period that individuals clean their feathers with their beak

(B) Behnke method to quantify ectoparasites

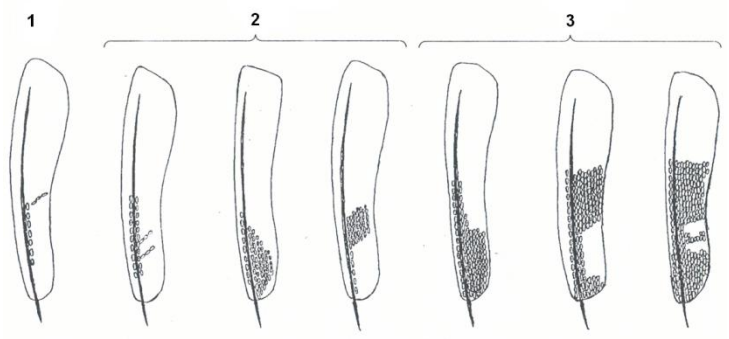

Figure 19 – Illustration of the Behnke method to quantify parasites. Briefly, the method consists in a classification of the quantity of parasites in the primary and secondary feathers of both wings, in a scale of 0 – 3, being: 0 without parasites, 1 – few parasites near ráquis, 2 – some near ráquis and dispersed in the barbs, 3 – dispersed all over the ráquis and barbs. Adapted from Behnke et al.(1999).

(C) Formulas used to calculate the colour variables adapted from Cardoso and Mota (2008)

BrTot (Total brightness) – mean values of the visible and UV light reflection (320-700 nm);

$$\text{BrTot} = \sum_{\lambda_{320}}^{\lambda_{700}} R_i / n_w$$

BrUV (ultraviolet brightness) – mean values of the UV reflection (320-415 nm);

$$\text{BrUV} = \sum_{\lambda_{320}}^{\lambda_{415}} R_i \ / \ n_w$$

Hue (hue) – wavelength reflection between the minimum and maximum values of reflections for 320-700nm;

$$\text{Hue} = \lambda_{[R\text{max} + R\text{min}]/2}$$

SatTot (Total Saturation) – Quotient difference between minimum and maximum reflection by the total brightness (320-700 nm);

$$\text{SatTot} = (R_{\text{max}} - R_{\text{min}}) / \text{BrTot}$$

SatUV (Ultraviolet Saturation) – Quotient difference between UV maximum reflectance (320-415 nm) and minimum (320-700 nm) for the total brightness (320-700 nm);

$$\text{SatUV} = (R_{\text{maxUV}} - R_{\text{min}}) / \text{BrTot}$$

(D) Mate Choice aviary

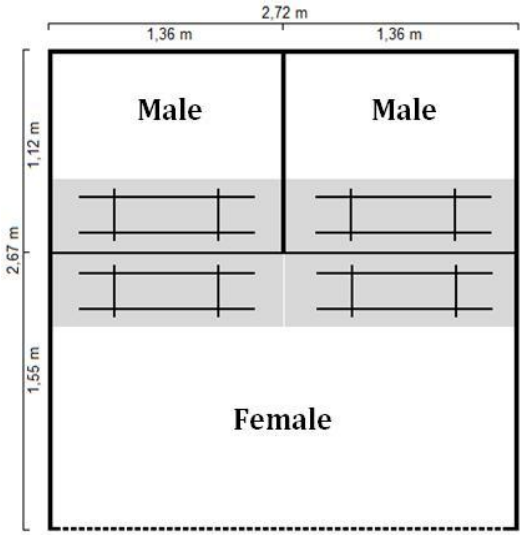

Figure 20 - Mate choice camera consisting in a three-compartment indoor aviary. The two male's compartments are separated each other by an opaque wall. Female's compartment is separated from male's compartment by a glass. Represented in grey are the "response areas", areas of association of the individuals, where are perches. In white are represented "out areas". During each trial, the males didn't have visual contact with each other and the female was only separated by glass from both males. The areas adjacent to each male were designated as the "response areas", where we used time spent by females in this interaction area as a measurement of female preference (Hill, 1990). In these areas there were perches, so we could be able to register other behaviours of interest such as billing and hopping.

Appendix II - Chapter 2 - Meaning of colouration and UV reflectance in mate choice of Serin

(A) - Sex, age morphometric data and ectoparasites

Females

								Beak			
Ring Nr.	**Date**	**Sex**	**Age**	**Weight**	**Right**	**Tail**	**Tarsus**	**Height**	**Length**	**Width**	**Parasites**
131	11-Fev	F	1	10.5	65	16.02	43.3	6.86	7	6.2	0
132	11-Fev	F	A	10.5	68	15.1	44	6.32	7.4	6.38	10
133	11-Fev	F	A?	9.75	68	15.2	47.74	6.2	7.34	6.2	2
134	11-Fev	F	A	8.75	70	15.92	46.6	6.3	6.94	6.27	8.5
135	11-Fev	F	A	11	66	15.6	44.92	5.88	6.5	6.18	10
136	11-Fev	F	A	9	65	15.54	45.26	5.62	6.8	6.88	8
141	11-Fev	F	A	11.5	69	15.65	48.64	6.24	7.28	6.18	3.5
144	25-Fev	F	1	11	70	15.22	47.02	6.2	6.2	6.66	10.5
145	25-Fev	F	1	10.5	68	15.72	46.78	6.22	6.88	6.64	5.5
146	25-Fev	F	1	12	67	16.06	46.38	6.62	6.98	6.48	14
147	25-Fev	F	1	10.5	69	15.54	45.98	6.18	7.12	6.18	3.5
167	22-Mar	F	1	11	65	13.7	43.02	5.82	7.28	6.4	3
173	22-Mar	F	1?	12	68	14	46.72	5.52	7.94	6.28	2.5
168	22-Mar	F	A	11.25	67	14.94	45.94	6.2	7.86	6.2	4.5
169	22-Mar	F	1	11	66	14.91	44.32	6.38	7.28	6.64	0
174	23-Mar	F	1	12	68	14.52	44.82	6.34	6.64	5.24	0
175	23-Mar	F	1	11.75	65	14.56	43.4	6.19	7.08	6.19	1.5
176	23-Mar	F	1	11.75	71	14.52	47.04	6.18	7.16	6.38	2.5
177	23-Mar	F	1	11	68	15.02	47.24	6.4	6.84	6.74	4

Males

								Beak				
Ring Nr.	**Date**	**Sex**	**Age**	**Weight**	**Right**	**Tail**	**Tarsus**	**Height**	**Length**	**Width**	**Parasites**	**Colouration Score**
142	11-Fev	M	A	10.25	73	49.12	15.68	6.18	6.82	6.32	7	-0.68181
143	17-Fev	M	A	11.25	71	50.42	15.08	6.2	7.6	6.2	3.5	0.57377
154	21-Mar	M	A	10.5	73	50.68	13.7	5.58	7.28	7.52	6	0.10821
156	22-Mar	M	1	11	68	45	15.55	6.48	7.6	6.2	3	2.25123
158	21-Mar	M	A	11.5	73	48.3	14.66	5.76	7.6	6.5	0	1.01802
159	21-Mar	M	A	10.5	73	46.36	15.48	6.42	7.37	6.2	3.5	-0.34601
160	22-Mar	M	A	11.25	73	52.02	14.68	6.28	7.1	6.7	1.5	0.15184
161	21-Mar	M	1	11.5	70	49.54	14.48	5.54	7.5	6.3	6	1.75947
162	22-Mar	M	A	11.25	74	50.34	14.38	6.56	7.06	6.44	3	-1.22498
163	22-Mar	M	A	10	70	48.6	13.58	5.52	6.68	6.28	3	-0.93227
164	22-Mar	M	A	11.75	72	47.04	14.56	6.08	6.68	6.72	5.5	-0.86579
165	21-Mar	M	A	10	69	47.36	14.73	6.18	7.04	6.2	5.5	-1.10519
166	21-Mar	M	A	11.5	69	49.14	14	6.64	7.34	6.44	1.5	-0.28837
180	29-Mar	M	1	10	73	49.83	14.18	6.18	7.18	6.92	8	0.3024
181	29-Mar	M	A	11.5	73	48.64	15.01	6.29	7.33	6.2	4.5	-0.83444
182	29-Mar	M	A	11.25	71	48.68	15.36	6.2	7.12	6.66	7	0.61962
183	29-Mar	M	1	11.5	71	47.25	15.53	6.28	6.66	6.21	4.5	-0.50571

(B) - Variables calculated from the spectrophotometry values, for the three sampled regions.

Region	Throat					Breast					Yield				
Variables → ↓ Males	tmb	tmbuv	th	ts	tsuv	bmb	bmbuv	bh	bs	bsuv	ymb	ymbuv	yh	ys	ysuv
142	13.0	6.8	529.4	1.6	0.6	12.2	8.1	551.4	1.2	0.6	28.0	18.6	551.8	1.1	0.5
143	15.9	9.0	499.3	1.3	0.4	19.1	12.4	572.8	1.3	0.7	30.2	20.5	573.7	1.1	0.4
154	19.8	10.7	552.2	1.4	0.5	21.3	10.8	552.3	1.5	0.5	24.6	17.4	498.0	1.1	0.5
156	27.7	13.2	565.9	1.6	0.7	17.9	12.2	510.3	1.3	0.7	32.7	24.4	510.2	1.2	0.7
158	23.5	10.6	571.1	1.6	0.5	16.7	12.3	550.7	1.1	0.6	24.8	21.3	557.7	0.8	0.6
159	19.8	9.8	572.0	1.6	0.6	14.8	7.1	551.8	1.6	0.6	27.3	17.5	565.9	1.1	0.5
160	18.0	9.0	573.9	1.6	0.5	13.5	8.9	552.0	1.2	0.6	27.5	22.1	497.8	0.8	0.4
161	22.2	12.3	514.2	1.5	0.6	20.1	13.5	551.3	1.2	0.6	28.0	22.7	514.0	0.7	0.4
162	17.8	7.6	515.0	1.6	0.5	11.7	6.9	550.5	1.3	0.5	26.9	16.1	574.5	1.2	0.5
163	20.0	9.0	552.7	1.5	0.4	18.1	9.8	552.4	1.4	0.6	24.2	11.6	572.3	1.5	0.4
164	16.4	6.0	551.9	1.7	0.4	18.4	10.3	551.9	1.5	0.6	25.5	15.3	551.6	1.3	0.6
165	16.7	8.1	553.7	1.5	0.5	16.5	8.5	566.4	1.5	0.5	21.0	14.4	566.4	1.2	0.6
166	16.1	8.6	553.8	1.4	0.5	17.8	8.3	551.4	1.6	0.5	31.3	21.2	573.0	1.0	0.4
180	22.0	11.3	529.8	1.6	0.6	18.4	9.4	551.5	1.5	0.6	26.4	17.5	559.5	1.1	0.5
181	17.8	7.9	554.4	1.7	0.5	13.7	6.8	566.4	1.6	0.6	27.5	17.1	552.5	1.3	0.6
182	20.0	11.1	573.4	1.5	0.5	18.9	10.4	572.8	1.5	0.6	33.2	20.0	565.7	1.3	0.6
183	17.4	8.8	514.8	1.5	0.5	17.6	9.1	572.0	1.5	0.5	27.3	16.8	551.9	1.3	0.6
Mean	19.1	9.4	545.7	1.5	0.5	16.9	9.7	554.6	1.4	0.6	27.4	18.5	549.2	1.1	0.5
Standard deviation	3.4	1.9	24.0	0.1	0.1	2.8	2.0	14.4	0.2	0.1	3.1	3.3	26.7	0.2	0.1
Coefficient of Variation	18.1	20.5	4.4	7.0	15.4	16.4	21.0	2.6	11.0	11.5	11.4	17.9	4.9	18.3	16.7

(C) - Matching pairs

(A)

Communalities

	Initial	Extraction
tmb	1,000	,785
tmbuv	1,000	,804
ts	1,000	,906
tsuv	1,000	,670
bmb	1,000	,917
bmbuv	1,000	,916
bs	1,000	,815
bsuv	1,000	,678
ymb	1,000	,800
ymbuv	1,000	,911
ys	1,000	,864
ysuv	1,000	,765

Extraction Method: Principal Component Analysis.

(B)

Component Matrix[a]

	Component			
	1	2	3	4
tmb	,747	,126	,348	-,300
tmbuv	,865	-,096	,209	,048
ts	-,143	,850	,122	-,384
tsuv	,470	,587	,006	,322
bmb	,472	-,760	,337	-,061
bmbuv	,801	-,407	-,040	-,327
bs	-,369	-,284	,679	,371
bsuv	,751	,099	,265	-,186
ymb	,514	,190	,136	,694
ymbuv	,830	,228	-,290	,294
ys	-,543	-,011	,751	,074
ysuv	,105	,446	,726	-,166

Extraction Method: Principal Component Analysis.
a. 4 components extracted.

(C)

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4,369	36,409	36,409	4,369	36,409	36,409
2	2,213	18,438	54,848	2,213	18,438	54,848
3	2,019	16,828	71,675	2,019	16,828	71,675
4	1,228	10,232	81,908	1,228	10,232	81,908
5	,977	8,143	90,050			
6	,362	3,013	93,063			
7	,332	2,770	95,833			
8	,293	2,445	98,279			
9	,187	1,560	99,838			
10	,012	,102	99,940			
11	,006	,051	99,991			
12	,001	,009	100,000			

Extraction Method: Principal Component Analysis.

Figure 21 – Output from SPSS, of a Principal Component Analysis, for the variables of colouration resulted from spectrophotometry analysis. (A) Variables used for the analysis (B) Matrix of the four significant components, being represented in the first one the saturation (s) and mean brightness (mb) and mean brightness UV (mbUV) (C) Total variance explained for each component, being significant the first 4 components.

Table XVII – Ascendant ordination of the males by **s**cores for the first factor of the principal component analysis that was used to separate the males into two groups: less colourful (M-) and the most colourful (M +) and (positive and negative values of PC1, respectively)

Males	Score
156	2.25123
161	1.75947
158	1.01802
182	0.61962
143	0.57377
180	0.3024
160	0.15184
154	0.10821
166	-0.28837
159	-0.34601
183	-0.50571
142	-0.68181
181	-0.83444
164	-0.86579
163	-0.93227
165	-1.10519
162	-1.22498

(D) - Protocols

Protocol 1 - UV-Blocking product preparation

Material:
Bottle of CDC Duck Preen oil (0.16g package + 9.42 g oil; total: 9.58 g)
Eusolex 9020 (Merck)
Flask for mixture
Balance (Max 60g)
Spoon
Microwave

Procedure:
1 – Prepare the balance. Tare the flask where the mixture is going.
2 – Prepare the product with 75% oil and 25 % of Eusolex:
- For each oil package (+-9.68 g) put 3.23 g of Eusolex
3 – Heat the mixture in the microwave for 40 seconds, at maximum potency.
4 – Wait for the mixture to cool down and apply.
5 – The mixture can be kept for several weeks. In case of being solidified, just reheat.

Protocol 2 – UV Manipulation

Material:
Cotton buds;
Isolation cages;
Spectrophotometer apparatus ;
Bottle of Duck preen oil;
Flask of UV blocking product prepared according UV-blocking protocol (protocol 1)

Procedure:
A. Day before the experiment:
1 – Prepare isolation cages. Identify the cages with the individual number and treatment type – control or UV-blocking.
2 – Turn on the spectrophotometer and prepare the apparatus.
3 –For each test, prepare two males. Measure the four colour zones – crown, bib, breast and abdomen - for each male before applying the products.
4 – Always at end of the day (around 17:30), apply the products in the coloured zones – crown, bib, breast and abdomen.
Male 1 – Control – Apply duck preen oil
Male 2 – Apply UV-blocking preparation– 25% of Eusolex AND 75% of Duck preen oil

B. Pair choice experiment day:
1 – Perform the mate choice experiment. Alternate the place of the individual with the UV-blocking treatment in the test chamber.
2 – Measure the tested individual by spectrophotometry, after the experiment(s).
3 – Place the individuals in an isolation cage for another day, with access to bathtubs.
4 – Place the individuals in their original cage the day after the experiment.

(E) Tests validated and used in the statistical analysis from Experiment I and II.

Table XIX - Experiment I. Male 1 in the left side, Male 2 in the right side. In grey the more colourful males.

Test Number	Male 1	Male 2	Female	Date	Date	Date
1	161	181	169	20/04		
2	166	156	131	20/04		
3	154	163	132	21/04	6/05	
4	165	182	146	21/04	7/05	
5	161	183	147	27/04	2/05	7/05
6	159	156	134	28/04	2/05	
7	143	181	167	28/04	3/05	9/05
8	163	182	170	29/04		
9	183	156	175	1/05		
10	143	159	174	1/05		
11	182	183	141	6/05		
12	162	160	145	7/05		

Table XX - Experiment II. Male 1 in the left side, Male 2 in the right side. In grey the more colourful males that suffered UV treatment, with a c, the males that were submitted to control treatment.

Test Number	Male 1	Male 2	Female	Date
1UV	181	161 c	169	10/05
2UV	156 c	166	131	10/05
3UV	163	154 c	132	10/05
4UV	182 c	165	146	10/05
5UV	183	161 c	147	11/05
6UV	156 c	159	134	11/05
7UV	181	143 c	167	11/05
8UV	182 c	163	170	11/05
9UV	156 c	183	175	12/05
10UV	159	143 c	174	12/05
11UV	160 c	162	145	12/05
12UV	164	158 c	171	13/05

Appendix III - Chapter 3 - Influence of plumage maintenance behaviour in Serin's mating behaviour

(A) - Sex, age, morphometric data and ectoparasites

Females

						Beak			
Ring Nr.	**Age**	**Weight**	**Right**	**Tail**	**Tarsus**	**Height**	**Length**	**Width**	**Parasites**
1	1	11.50	68	16.86	44.33	6.54	7.18	6.85	8
4	A	11.50	67	16.31	44.82	6.15	7.15	6.64	8
5	1	12.50	68	18.00	44.56	6.55	8.09	6.68	13
6	1	12.00	68	17.13	45.17	6.04	7.26	6.54	11
7	1	12.50	68	20.46	41.24	6.57	7.95	6.83	5.5
8	1	13.50	67	17.84	44.84	6.56	7.64	6.96	7
77	A	11.50	67	17.94	42.72	6.14	7.44	6.24	2
80	A	10.50	65	16.04	44.14	6.70	8.88	6.10	9
83	1	11.00	68	18.58	46.58	6.04	8.06	6.30	22
87	1	11.00	69	16.56	46.14	6.04	7.14	6.48	17
88	1	12.25	71	14.67	44.14	6.38	7.66	6.18	7
89	1	11.75	68	14.24	46.14	5.90	7.06	6.52	16
91	1	12.00	68	16.42	44.38	5.90	7.08	5.74	2
92	A	12.25	69	16.58	45.48	7.00	7.82	6.18	17
95	A	11.00	70	18.24	48.08	5.80	7.32	6.24	16
99	A	11.75	69	15.75	50.12	6.02	7.06	6.58	3.5
100	A	11	67	15.98	47.14	6.8	6.92	6.04	3.5
101	1	11.5	68	15.65	47.38	5.82	6.76	6.12	11
103	A	10.5	70	15.96	48.46	5.4	6.67	6.66	4
104	1	12	67	16.2	43.41	6.43	7.64	6.54	8
105	1	11	66	15.3	43.45	6.25	6.39	6.06	6
106	A	11.5	67	15.94	43.88	6.63	7.6	6.08	9.5

Males

						Beak				
Ring	Age	Weight	Right	Tail	Tarsus	Height	Length	Width	Parasites	Colouration Score
12	A	11.50	73	47.84	17.17	6.12	7.64	6.32	10.5	-1.18072
13	A	12.25	71	48.00	15.54	6.26	8.08	6.72	7	-0.25555
14	1	11.50	68	49.30	16.41	6.05	7.91	6.53	13.5	0.73329
15	A	10.50	69	49.37	16.53	6.03	7.98	6.12	20	0.84386
96	A	12.50	70	46.55	18.72	6.38	7.12	6.34	6.5	-0.4764
97	1	11.25	70	48.06	14.64	6.04	6.73	5.52	4.5	-0.05525
98	A	11.00	68	46.94	16.16	6.84	7.30	5.58	11.5	-0.81808
100	1	11.50	71	45.66	16.60	6.20	7.56	6.97	8.5	0.11279
107	1	13	72	50.6	15.88	6.4	7.18	6.75	9	0.11083
109	A	12.5	72	50.56	16.32	7.08	7.77	6.38	6.5	-0.49589
111	A	11	71	47.74	15.8	6.74	7.38	7.14	10.5	1.21378
115	1	10.5	68	43.08	15.2	5.76	6.22	5	0.5	0.84702
116	A	14	69	51.31	15.2	6.06	6.71	5.85	2.5	-1.96841
118	A	14	71	46.79	15.29	6.22	6.72	6.92	6	-0.94324
119	1	13	69	47.04	15.37	5.5	6.5	5.94	4	2.72112
120	A	13	70	47.92	15.99	5.69	7.24	6.53	2.5	0.57683
121	1	12	67	43.38	14.95	5.36	6.77	6.02	6	-0.75662
123	A	11	69	48.58	15.79	6.42	7.35	6.32	4.5	0.75954
124	A	11.25	70	44.94	17.44	6.00	7.79	6.39	12	-1.71797
125	1	12.00	69	45.26	15.62	6.48	8.58	7.06	8	-0.12506
126	A	11.25	71	49.30	18.62	6.08	7.80	6.65	16.5	1.13499
130	A	10.75	69	47.34	15.94	5.58	7.16	6.54	15.5	0.28795
135	A	12.25	72	47.55	17.39	5.77	8.11	7.42	6	-1.15039
137	1	11.50	72	47.00	17.26	6.16	8.21	6.55	9	1.74122
138	1	11.50	68	44.98	17.24	6.14	7.54	6.30	12	0.27359
139	1	12.50	69	49.29	17.35	6.23	7.57	7.04	10	-0.82313
140	1	11.50	70	47.02	18.38	5.73	7.74	6.06	9	1.41645
141	A	11.50	73	46.24	19.50	6.13	7.46	6.17	7.5	0.34262
142	A	12.50	74	49.35	16.59	6.28	7.26	6.25	13.5	0.11717
143	A	10.75	69	48.48	16.93	6.02	7.42	7.04	18	-0.55292
145	1	11.75	70	48.98	17.03	5.95	8.23	6.08	28	-0.05985
147	A	10.75	69	47.03	17.14	5.80	8.00	6.44	17.5	0.08535
148	A	11.25	74	49.64	16.60	6.04	8.55	6.09	15.5	0.1356
149	1	11.00	72	45.96	18.14	6.44	7.96	6.86	20.5	0.36456

(B) Variables calculated from the spectrophotometry values, for the three sampled regions.

Region	Forehead					Throat				
Variables → ↓ Males	mb	mbuv	h	s	suv	mb	mbuv	h	s	suv
12	5.5	3.2	553.5	1.4	0.5	8.7	4.9	554.2	1.7	0.8
13	6.2	2.7	552.8	1.6	0.4	12.8	7.4	552.5	1.6	0.8
14	11.8	5.9	552.1	1.5	0.6	14.3	6.8	552.6	1.6	0.6
15	8.2	4.4	551.9	1.5	0.5	16.2	10.0	552.7	1.3	0.6
96	8.4	4.5	554.2	1.4	0.5	10.5	8.3	515.9	1.3	0.9
97	12.4	4.0	484.0	2.7	0.2	13.6	8.2	552.9	1.3	0.5
98	11.9	4.9	553.3	1.7	0.5	13.1	6.3	552.4	1.6	0.6
100	10.1	4.4	549.7	1.6	0.5	13.4	7.0	552.1	1.6	0.7
107	10.0	4.6	551.7	1.5	0.4	12.1	6.7	515.8	1.6	0.7
109	7.0	3.1	552.8	1.6	0.4	12.1	7.2	551.8	1.4	0.7
111	15.3	6.6	552.1	1.7	0.6	16.4	9.8	514.0	1.5	0.7
115	11.3	5.4	553.0	1.6	0.5	15.6	9.1	552.3	1.4	0.6
116	7.5	4.0	551.5	1.4	0.5	10.9	6.6	497.6	1.5	0.7
118	7.7	3.3	501.3	1.6	0.4	12.7	6.8	515.4	1.6	0.7
119	9.2	5.1	515.2	1.5	0.6	20.8	13.2	515.6	1.4	0.7
120	11.6	6.2	513.5	1.5	0.5	15.9	9.3	497.1	1.5	0.7
121	9.5	4.6	551.1	1.6	0.5	12.2	6.0	551.0	1.7	0.6
123	15.1	6.1	551.8	1.7	0.5	17.6	9.5	497.8	1.6	0.7
124	12.9	2.4	472.5	3.4	0.1	8.6	4.2	551.8	1.6	0.7
125	13.5	5.3	552.5	1.7	0.5	13.6	7.4	516.0	1.6	0.7
126	11.6	4.0	552.4	1.8	0.4	15.3	7.9	552.2	1.6	0.7
130	13.8	6.7	513.6	1.5	0.5	13.6	9.3	514.2	1.3	0.8
135	13.3	5.2	552.5	1.7	0.5	9.6	4.5	554.2	1.6	0.6
137	9.9	4.7	551.9	1.6	0.6	16.9	10.3	552.3	1.4	0.7
138	7.8	4.1	555.3	1.3	0.3	12.3	6.5	571.8	1.6	0.6
139	8.4	3.7	551.8	1.6	0.5	10.9	5.9	553.7	1.5	0.7
140	8.5	4.7	551.6	1.3	0.4	16.2	9.2	495.8	1.4	0.3
141	9.6	5.1	553.0	1.4	0.5	13.0	8.2	552.3	1.4	0.7
142	10.6	4.4	552.9	1.6	0.3	14.6	7.1	572.5	1.6	0.6
143	8.1	3.5	551.9	1.6	0.4	13.2	7.2	552.1	1.5	0.7
145	6.6	3.3	551.4	1.5	0.4	13.1	6.5	552.4	1.5	0.5
147	6.9	2.6	555.5	1.8	0.4	13.5	7.0	553.4	1.5	0.6
149	11.0	4.1	555.3	1.7	0.4	15.9	9.0	573.0	1.4	0.6
Mean	10.0	4.4	543.0	1.7	0.4	13.6	7.7	539.7	1.5	0.7
Standard deviation	2.6	1.1	21.8	0.4	0.1	2.6	1.8	23.6	0.1	0.1
Coefficient of Variation	26.0	25.1	4.0	23.9	22.4	19.2	24.0	4.4	7.2	14.7

Region	Breast					Yield				
Variables → ↓ Males	mb	mbuv	h	s	suv	mb	mbuv	h	s	suv
12	9.8	6.3	551.7	1.5	0.8	20.6	12.2	573.4	1.4	0.6
13	13.4	8.4	570.3	1.4	0.7	20.9	11.7	551.7	1.4	0.6
14	14.9	8.6	552.6	1.5	0.7	23.7	15.8	557.4	1.2	0.6
15	15.4	9.1	551.8	1.3	0.5	18.5	12.5	514.9	1.3	0.7
96	10.8	6.6	554.2	1.5	0.8	17.4	11.4	515.2	1.4	0.7
97	11.4	6.7	553.5	1.4	0.7	22.1	11.8	496.4	1.5	0.4
98	6.7	3.6	516.6	1.6	0.7	21.0	11.1	552.4	1.4	0.6
100	12.3	7.6	515.7	1.5	0.8	23.2	14.2	515.5	1.3	0.6
107	10.2	7.3	515.4	1.4	0.8	19.2	17.5	513.6	1.0	0.7
109	7.6	5.2	498.3	1.4	0.7	20.2	12.8	515.6	1.4	0.7
111	13.0	9.2	514.1	1.4	0.8	22.2	13.1	515.0	1.5	0.7
115	11.3	7.6	516.0	1.4	0.8	22.9	14.4	553.5	1.3	0.6
116	4.5	2.5	499.0	1.9	0.7	12.2	8.1	497.7	1.5	0.8
118	7.2	4.8	498.8	1.7	0.9	19.0	13.4	513.8	1.3	0.7
119	18.3	14.9	514.4	1.2	0.9	24.2	17.2	513.7	1.4	0.9
120	14.7	7.0	515.1	1.6	0.6	20.6	12.5	516.4	1.4	0.6
121	6.2	3.2	552.5	1.6	0.6	19.4	14.0	514.4	1.1	0.6
123	10.4	7.2	497.7	1.3	0.6	20.8	12.7	497.5	1.3	0.6
124	10.3	5.7	554.1	1.5	0.7	17.6	11.8	513.8	1.3	0.7
125	12.3	6.6	551.2	1.5	0.6	19.3	11.4	514.8	1.4	0.6
126	15.8	10.7	552.1	1.3	0.7	22.6	19.1	515.1	0.9	0.5
130	14.6	8.0	496.5	1.7	0.4	16.2	11.4	514.2	1.2	0.6
135	6.0	3.1	554.0	1.6	0.7	20.6	13.0	514.2	1.3	0.6
137	18.9	13.0	551.6	1.2	0.7	20.1	16.6	516.3	1.0	0.7
138	15.5	10.1	572.6	1.3	0.7	22.3	13.6	553.3	1.4	0.7
139	7.9	4.9	552.5	1.4	0.7	18.2	12.7	553.6	1.3	0.7
140	12.3	8.6	515.1	1.3	0.7	27.5	17.6	514.0	1.4	0.7
141	13.4	8.6	515.0	1.4	0.8	19.6	13.6	515.3	1.2	0.6
142	15.0	8.1	552.4	1.5	0.6	20.5	13.2	515.1	1.4	0.7
143	12.5	7.1	552.0	1.5	0.7	24.8	8.6	483.8	2.5	0.3
145	11.7	7.3	522.9	1.3	0.6	17.8	12.9	523.9	1.0	0.5
147	17.2	9.2	514.1	1.6	0.7	23.3	14.0	515.8	1.3	0.6
149	11.1	6.0	551.8	1.5	0.7	23.2	13.5	546.1	1.3	0.5
Mean	11.9	7.4	533.2	1.5	0.7	20.7	13.3	522.3	1.3	0.6
Standard deviation	3.6	2.6	23.8	0.1	0.1	2.9	2.4	20.6	0.3	0.1
Coefficient of Variation	30.4	35.8	4.5	10.0	14.8	13.9	17.9	3.9	19.2	18.0

(C) Matching pairs - PCA

(A)

Communalities

	Initial	Extraction
fmb	1,000	,976
fmbuv	1,000	,890
fs	1,000	,914
fsuv	1,000	,834
tmb	1,000	,874
tmbuv	1,000	,974
ts	1,000	,747
tsuv	1,000	,894
bmb	1,000	,858
bmbuv	1,000	,941
bs	1,000	,740
bsuv	1,000	,863
ymb	1,000	,925
ymbuv	1,000	,924
ys	1,000	,893
ysuv	1,000	,723
COROA	1,000	,881
PEITO	1,000	,927

Extraction Method: Principal Component Analysis.

(B)

Component Matrix[a]

	Component						
	1	2	3	4	5	6	7
fmb	,204	,213	-,431	,743	-,016	-,004	,391
fmbuv	,493	,524	-,427	,266	-,110	-,255	,208
fs	-,315	-,327	-,051	,586	,118	,537	,243
fsuv	,385	,556	-,195	-,346	-,093	-,420	,183
tmb	,889	,080	-,183	,053	,145	,018	-,138
tmbuv	,869	,217	-,235	-,147	-,006	,294	-,089
ts	-,373	-,017	,386	,201	,051	-,590	,260
tsuv	-,192	,346	,108	-,338	-,490	,179	,583
bmb	,746	-,434	-,098	-,113	-,273	,077	,097
bmbuv	,851	-,316	,117	-,153	-,189	,155	,138
bs	-,728	,375	-,234	-,025	-,081	-,023	-,084
bsuv	,007	,114	,675	-,196	,392	,202	,402
ymb	,524	-,407	,043	-,044	,615	-,232	,221
ymbuv	,664	-,161	,574	,285	,001	-,211	-,034
ys	-,238	-,042	-,492	-,478	,503	,184	,277
ysuv	,206	,441	,524	,064	-,381	,247	-,023
COROA	,128	,825	,213	,074	,317	,164	-,069
PEITO	-,196	-,820	-,158	-,106	-,362	-,133	,178

Extraction Method: Principal Component Analysis.

a. 7 components extracted.

(C)

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4,935	27,414	27,414	4,935	27,414	27,414
2	3,093	17,183	44,597	3,093	17,183	44,597
3	2,110	11,721	56,318	2,110	11,721	56,318
4	1,674	9,299	65,617	1,674	9,299	65,617
5	1,578	8,769	74,386	1,578	8,769	74,386
6	1,305	7,253	81,639	1,305	7,253	81,639
7	1,084	6,023	87,662	1,084	6,023	87,662
8	,710	3,943	91,605			
9	,488	2,714	94,319			
10	,326	1,810	96,128			
11	,278	1,545	97,674			
12	,158	,879	98,553			
13	,133	,738	99,290			
14	,053	,293	99,583			
15	,033	,183	99,766			
16	,023	,129	99,895			
17	,014	,080	99,974			
18	,005	,026	100,000			

Extraction Method: Principal Component Analysis.

Figure 22 – Output from SPPSS, of a Principal Component Analysis, for the variables of colouration resulted from spectrophotometry analysis. (A) Variables used for the analysis (B) Matrix of the seven significant components, being represented in the first one the saturation (s) and mean brightness (mb) and mean brightness UV (mbUV) (C) Total variance explained for each component, being significant the first 7 components.

Appendix IV - Chapter 4 - Personality and Mate Choice in Serin

(A) Principal component Analysis to score Personality

Communalities (A)

	Initial	Extraction
Tonic Immobility	1,000	,413
NewObjectActivity2	1,000	,875
New Object - Latency to object	1,000	,734
New Object - Latency to food	1,000	,849

Extraction Method: Principal Component Analysis.

Component Matrix[a] (B)

	Component	
	1	2
Tonic Immobility	,523	,373
NewObjectActivity2	,012	,935
New Object - Latency to object	,852	,091
New Object - Latency to food	,860	-,330

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

Total Variance Explained (C)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	1,740	43,511	43,511	1,740	43,511	43,511
2	1,131	28,263	71,774	1,131	28,263	71,774
3	,823	20,575	92,349			
4	,306	7,651	100,000			

Extraction Method: Principal Component Analysis.

Figure 23 – Output, from SPSS, of a Principal Component Analysis, for the variables of behaviour resulted from the behavioural tests. (A) Variables used for the analysis (B) Matrix of the two significant components, being represented in the first one by negative values, activity and exploration and positive values indicate boldness (C) Total variance explained for each component, being the first two components significant.

(B) Personality score

Females		Males	
Individual	**Score**	**Individual**	**Score**
131	0.75409	**142**	1.69272
132	1.23195	**143**	-1.06309
134	0.45176	**154**	1.05992
136	1.70221	**156**	-.26181
141	0.82571	**159**	-1.46278
144	0.99877	**160**	-1.46182
145	-0.82750	**161**	.70620
146	-0.28962	**162**	-1.25134
147	-0.74312	**163**	.53236
167	-0.38797	**164**	-1.22426
169	0.43917	**165**	-1.29502
170	-0.25006	**166**	-1.43240
172	0.11061	**180**	-.32742
174	0.48030	**181**	1.69589
175	0.63824	**182**	.32737
177	-0.09787	**183**	-1.27120

(C) Effect of morphometric parameters, colouration and parasites

Males

Correlations

		Age	Weight	Right Wing	Tail	Tarsus	Beak Height	Beak Length	Beak Width	Parasites	Colouration Score
Personality score	Corr. Coef.	0.000	0.192	-0.152	0.115	-0.050	0.340	-0.212	-0.118	-0.470	-0.153
	Sig. (2-t)	1.000	0.477	0.575	0.672	0.854	0.198	0.430	0.663	0.066	0.572
	N	16	16	16	16	16	16	16	16	16	16

*. Correlation is significant at the 0.05 level (2-tailed).

Females

Correlations

		Weight	Right Wing	Tail	Tarsus	Beak Height	Beak Length	Beak Width	Parasites
Personality score	Correlation Coefficient	-0.023	0.329	0.363	0.376	0.086	0.007	-0.048	0.181
	Sig. (2-tailed)	0.939	0.251	0.203	0.185	0.771	0.982	0.869	0.535
	N	16	16	16	16	16	16	16	16

**. Correlation is significant at the 0.01 level (2-tailed).