LINKING COURTSHIP BEHAVIOUR, COLOUR PERCEPTION AND MATE
CHOICE DECISIONS IN PEAFOWL

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ABSTRACT

Despite a long history of study showing that male courtship signals influence female mate choice in many species, we lack a good understanding of how females choose. What are the mechanisms of mate choice, and how do these mechanisms shape the evolution of courtship signals and traits? In this thesis, I use the peacock’s iridescent eyespots to link signal perception with female mate choice decisions and the behaviours males use during courtship. I begin by investigating how a peacock’s eyespot colours influence his mating success, using models of avian colour vision and measurements of eyespot plumage colours taken at light angles that mimic the way the feathers are displayed during courtship. My results suggest that a substantial portion of the variation in peacock mating success can be explained by these plumage colours, demonstrating that signal function is best understood by considering the context in which signals are presented. Next, I examine how females choose to visit different males for courtship. I show that a female’s familiarity with a male as a result of previous courtship encounters affects how she responds to his signals, including his eyespot colours. Lastly, I examine the visual effects of the peacock’s iridescent eyespot colours under different light conditions, and show that typical male courtship behaviours might enhance the eyespots in a way that influences female choice. I also find evidence that light conditions and female sensory biology together may have shaped the evolution of the eyespot colours in two species of peafowl. Overall, the results of this thesis demonstrate that by understanding how animals perceive colour signals, we can gain a better understanding of the function of behaviour on both sides of the courtship signaling exchange.
CO-AUTHORSHIP

This thesis follows the Manuscript Format guidelines laid out by the Department of Biology at Queen’s University.

Chapter 2 was co-authored with my supervisor Robert Montgomerie, and has been submitted for publication in the journal *Behavioral Ecology*. Dr. Montgomerie contributed to the conceptual design, statistical analyses, and writing of that chapter. Two anonymous reviewers also provided a wealth of useful comments on an earlier version of Chapter 2. Dr. Montgomerie also contributed to the concept and design of Chapters 3 and 4, and we anticipate publishing these studies as co-authors.
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CHAPTER 1. The mate choice decision

Before he proposed to his wife, Charles Darwin – aged 29 – drew up a list of pros and cons under the headings “Marry” and “Not Marry” – “This is the Question” (Darwin 1838). Upon finding the scales tipped in favour of marriage, he made yet another list to weigh the question of “When? Soon or Late”. Years later, after he had developed the theory of natural selection (Darwin 1859), Darwin turned to the question of how animals decide to mate. Noting that females often have strong preferences or aversions for certain males, he argued that these preferences could lead to the evolution of extravagant traits that seem to fly in the face of natural selection, like the peacock’s tail (Figure 1.1; Darwin 1871). He called this process sexual selection to distinguish it from his earlier theory. Although Darwin described females deliberately choosing their mates, he was careful to add that they need not apply the same kind of thorough analysis that he did when it came to his marriage. On the female bird he wrote:

“It is not probable that she consciously deliberates; but she is most excited or attracted by the most beautiful, or melodious, or gallant males. Nor need it be supposed that the female studies each stripe or spot of colour; that the peahen, for instance, admires each detail in the gorgeous train of the peacock – she is probably struck only by the general effect.” (Darwin 1871 v. 2: 123)

It took more than a century for sexual selection to gain wide acceptance, but today it has been reinforced by the evidence that females (and males) of many species prefer mates with certain phenotypic traits (reviewed in Andersson 1994). We also have several
different theoretical models for the adaptive benefits of mate choice and how such choice might evolve, as well as an explanation for why females are generally the choosier sex (reviewed in Kokko et al. 2006). Yet despite rapid growth in the study of sexual selection over the past 30 years, we know relatively little about the cognitive process of mate choice to which Darwin alluded (Gibson and Langen 1996; Jennions and Petrie 1997).

Understanding how mate choice occurs is an important goal for evolutionary biology, since the underlying mechanisms of choice can influence how sexual selection shapes male traits (Gibson and Langen 1996; Jennions and Petrie 1997; Bateson and Healy 2005; Kokko et al. 2006; Laland et al. 2011). Moreover, as biologists have begun to appreciate the importance of choice in other social interactions – such as cooperation and parental care – there is an increasing awareness that social choice drives the evolution of traits and behaviours beyond those used in courtship (Lyon and Montgomerie 2012). To what extent are mate choice, parental favouritism, and cooperative affinities based on common underlying mechanisms, and what are the evolutionary implications?

As Darwin’s marriage list illustrates, the process of choosing a mate might be considered an information-processing task (Miller and Todd 1998): courtship may involve perceiving and assessing a variety cues, remembering this information, and integrating it over time to come to a decision (Bateson and Healy 2005). These activities may draw heavily on the cognitive resources of animals, especially where females consider multiple potential mates before making a choice (Wittenberger 1983; Bateson and Healy 2005). Or they may not – honeybee swarms (Apis mellifera) can compare several different nest
sites using relatively simple mechanisms (Seeley et al. 2012), and even single-celled slime moulds (*Physarum polycephalum*) can compare foraging patches without the use of a brain (Latty and Beekman 2011). As Darwin noted, we easily attribute cognitive complexity to animals for behaviours that may not require it (Darwin 1871 v. 1: 46).

In this thesis, I aim to clarify how females use the peacock’s train ornament to choose a mate (Figure 1.1). In the following sections, I review previous work on the cognitive processes underlying mate choice, including learning, memory, perception, and decision-making. Following this, I describe the goals of this thesis and provide additional background on the study system and methods used.
Figure 1.1 A peacock (*Pavo cristatus*) displaying his erect train.
1.1 LEARNING AND MEMORY

Some of the earliest work on mate choice from a cognitive perspective touched on the question of how animals learn to discriminate potential mates from non-mates, such as heterospecific or same-sex individuals. In his pioneering work on imprinting in birds – a form of learning where exposure to certain cues early in development can lead to life-long preferences or aversions – Konrad Lorenz (1935) realized that parental traits often provide the cues that allow offspring to recognize potential mates later in life.

Since then, experimental studies of birds have demonstrated that sexual imprinting occurs in a variety of species, and that its effects can be persistent and strong (e.g., Cooke et al. 1972; Immelman 1972; Bateson 1978). Evidence that young quail (*Coturnix japonica*) learn to avoid their rearing companions – most likely to avoid inbreeding (Bateson 1978) – led to the suggestion that imprinting could also cause females to prefer males with novel traits, potentially promoting the evolution of novel traits through mate choice (ten Cate and Bateson 1988). Experimental studies demonstrating that birds can also learn to prefer mates with the same novel traits borne by their parents, such as snow geese (*Chen caerulescens*) painted unnaturally pink (Cooke et al. 1972), or zebra finches (*Taeniopygia guttata*) wearing artificial paper crests, lend further support to this idea (Witte and Sawka 2003; Burley 2006).

Imprinting might also lead to sexual selection for elaborate traits in other ways. For instance, when an animal learns a positive association for one stimulus and a negative association for another along a single sensory dimension, it will subsequently respond
even more strongly to more extreme cues along that dimension. This phenomenon is known as peak shift. Weary et al. (1993) suggested that peak shift could lead to mate preferences for exaggerated sexual dimorphism in species where offspring imprint on the traits of both sexes. In the zebra finch, for example, males have red beaks whereas female beaks are orange; a peak shift effect could cause females to prefer the reddest and males the yellowest beaks. Consistent with this scenario, Weisman et al. (1994) showed that young females that lack sexually dimorphic parents fail to acquire a preference for red-beaked males as adults. More recently, ten Cate et al. (2006) found direct evidence that peak shift influences beak colour preferences in an experiment where male zebra finches were raised by parents with either normal or sex-reversed beak colours. At maturity, the males were offered a choice of eight different females with beak colours altered to display colours along a scale from red to yellow. The males in this experiment consistently preferred those females with more exaggerated versions of their mother’s beak colour, reinforcing the importance of peak shift in the development of sexual preferences (ten Cate et al. 2006).

Short-term effects of learning on mate choice are also common. Bakker and Milinski (1991) coined the phrase “previous male effect” to describe situations where a female’s response to a current male depends on the traits of those she has previously encountered, and they found evidence for this effect in experimental studies of stickleback fish (Gasterosteus aculeatus). Since then, the effect has also been demonstrated in experimentally controlled encounters between females and different males in a number of
species (e.g., Gabor and Halliday 1997; Wagner et al. 2001; Bailey and Zuk 2009; Izzo and Gray 2011).

We also know from research on individual recognition that many species can identify and remember specific group mates based on unique visual, vocal and chemical features (Tibbetts 2002; Tibbetts and Dale 2007), and that some animals can retain these memories for months or even years (Godard 1991; Insley 2000; Boeckle and Bugnyar 2012). Thus, it seems likely that memory for specific individuals and their traits could affect courtship and mate choice in the short- and long-term.

1.2 PERCEPTION

Courtship is fundamentally an exchange of signals, and as with many communication systems, there is often a close fit between the signal and the sensitivity of the receiver. Some of the first evidence for this came from the study of the calls used by male frogs to attract mates: Ryan and Wilczynski (1988) found a significant difference in the call frequencies of males from two neighbouring cricket frog (Acris crepitans) populations, and they showed that the inner ear structures of females from each population were most sensitive to the call frequencies of local males. Other neurophysiological studies of auditory perception in fish (Sisneros and Bass 2003; Sisneros et al. 2004) and birds (Lucas et al. 2007) suggest that there may often be a close match between acoustic sensitivity and courtship song. This could result from selection on females to tune in to male signals, or from selection on the signal itself, or both.
In their studies of cricket frogs, Ryan and coauthors have also shown that females prefer the songs of males from their own population as a result of these differences in female auditory sensitivity (Ryan and Wilczynski 1988; Ryan et al. 1992). The growing appreciation that perceptual systems could affect mate choice in cricket frogs and other species, including the túngara frog (*Engystomops pustulosus*), led to the suggestion that sensory biology might be the source of sexual selection for exaggerated traits (Ryan et al. 1990; Ryan and Rand 1990). According to the sensory bias model, mate preferences are the byproduct of pre-existing features of animal sensory systems, which might explain the evolution of mate choice in the absence of other adaptive benefits of choice (reviewed in Fuller et al. 2005).

Although we lack critical tests of the sensory bias model for the exaggeration of male traits (Fuller et al. 2005; Kokko et al. 2006), the fact that male ornaments often appear to mimic food items provides suggestive evidence for this theory (e.g., Proctor 1991; Rodd et al. 2002; Garcia and Ramirez 2005). For instance, male *Neumania papillator* water mites vibrate their legs with the same frequency as females’ typical copepod prey (Proctor 1991). Similarly, in several species of Goodeinae fishes, males have a conspicuous yellow band on their tails that resembles a quivering worm (Garcia and Ramirez 2005). In both cases, females perform similar behaviours in response to male signals as they do when presented with prey items (i.e., clutching in *Neumania* water mites and biting in Goodeinae fishes). Additional support for the theory comes from comparative phylogenetic evidence that female preferences may have evolved before preferred male traits in some lineages (e.g., Basolo 1990; Garcia and Ramirez 2005).
In addition to suggesting the origin for mate preferences, sensory biology might also drive selection for increased efficacy of courtship signals (Guilford and Dawkins 1991; 1993). There is considerable evidence for this in the study of animal signals: for example, male *Phylloscopus* warblers living in darker habitats tend to have brighter body colour patches (Marchetti 1993), perhaps to enhance conspicuousness when courting females. Similarly, males of several Neotropical bird species perform their courtship displays in locations and at times of day that would maximize the contrast of their plumage against the visual background (Endler and Théry 1996; Heindl and Winkler 2003). Because selection for effective communication in different habitats can lead to signal divergence – a process known as sensory drive (Endler 1992; 1993) – it might also contribute to reproductive isolation and speciation where the divergent signals play an important role in courtship and mate choice (Boughman 2002).

Although most research on sensory drive has focused on signal divergence as a result of detectability in different habitats, other higher-level cognitive processes may also be involved. For instance, swordtail characin (*Corynopoma riisei*) are tropical fish where males court females with a flag-like opercular ornament that resembles the females’ insect prey. Recent studies indicate that ornament morphology in characin fishes has diverged in different habitats, such that male ornament shapes match the shapes of locally common insect prey. Furthermore, female preferences in these fishes are the result of experience with different prey types (Kolm et al. 2012). Thus, learning can also influence signal divergence under sensory drive.
An issue that we know relatively little about is how females discriminate among competing male signals. In túngara frogs \textit{(Engystomops pustulosus)} – a species in which males gather in choruses to attract females with their calls – females prefer more complex calls with “chuck” notes added on the end (Rand and Ryan 1981). Recently, Akre et al. (2011) investigated how females discriminate between the sequential calls of different males, and found that their discrimination abilities were consistent with Weber’s law. This law is a psychophysical principle stating that perception depends on relative, not absolute, differences between stimuli. Thus, as male calls become more exaggerated with a greater number of chucks added on the end, females require larger differences in the total number of chucks to discriminate between different calls. If Weber’s law applies broadly to mate choice, it would imply that perceptual abilities may limit the extent of trait exaggeration under sexual selection. Interestingly, Akre and Ryan (2010) found evidence for another mechanism in túngara frogs that may counteract this effect: additional chucks seem to improve females’ ability to retain a calling male’s location in working memory. Thus, cognitive mechanisms have the potential to both enhance and constrain the exaggeration of sexually selected traits.

In spite of a long history of work on model systems in neuroethology and comparative psychology, we are only beginning to understand how many animal perceptual systems work. There are no doubt countless examples of courtship signals exploiting female senses through mechanisms that we cannot even recognize, in addition to those that we are beginning to understand. As an illustration, Endler et al. (2010) recently showed that
great bowerbird (*Chlamydera nuchalis*) males arrange the stones on their display courts according to size, in a manner that creates an optical illusion for human observers. Kelley and Endler (2011) have also shown that female bowerbirds prefer this arrangement – but so far we can only guess at how this alters what the female birds perceive.

### 1.3 INFORMATION-GATHERING AND DECISION-MAKING

In some species, females visit a number of different males sequentially prior to mating, a process that has been characterized as an attempt to sample potential mates (Janetos 1980). How do females gather information about the males they encounter, and use this information – past and present – to inform their mate choice decisions?

Assuming that females sample in order to evaluate males and ultimately arrive at a choice, Janetos (1980) took the approach of modeling the fitness outcomes of a variety of simple decision-making algorithms as a function of the number and quality of males encountered. His aim was to determine which strategy would perform best under different conditions. Since then, a number of authors have taken a similar approach to modeling how various decision rules perform under different scenarios (e.g., Real 1990; Dombrovsky and Perrin 1994; Luttbeg 1996). In general, these theoretical decision rules can be grouped into two general classes: “threshold-based” mechanisms where females judge males against an internal standard, versus “best-of-*n*” or “sample-based” mechanisms where females visit and compare a number of different potential mates before returning to one for mating. Researchers studying mate choice in the lab and field have often attempted to compare their observations with simplified threshold- and
sample-based mechanisms from the theoretical literature. These studies are critical to understanding how animals make these decisions, but the results have been equivocal with respect to the decision-making mechanisms involved (Valone 1996; Wiegmann et al. 1996). This is in part because different decision rules can easily lead to the same behaviour, depending on various assumptions. Moreover, there may often be considerable variation between individual females in other factors that may affect their behaviour.

A question that is often overlooked is whether mate sampling necessarily represents an adaptive strategy at all, as has been generally assumed. Repeated courtship interactions might not part of a larger goal-directed sequence at all. It is also possible that these repeated interactions might be maladaptive for females, in that the costs involved may not outweigh the benefits. For example, it is theoretically possible for male traits to evolve that are increasingly effective at attracting female viewers to their detriment (e.g., Holland and Rice 1998; Arnqvist 2006). In theory, such manipulative courtship rituals can potentially evolve even in monogamous species (Wachtmeister and Enquist 2000), where courtship has long been assumed to have a mutually beneficial function.

An alternative approach is to view the mate sampling process as a series of choice decisions: females have the opportunity to approach different males, to engage in escalating bouts of courtship with them, and to accept or reject their copulation attempts on repeated occasions. Perception, attention, discrimination, and memory are involved at each step – so a better understanding of choice may require details of how these cognitive
processes function in natural courtship interactions (Gibson and Langen 1996). To date, only a handful of studies of mate-sampling behaviour have explicitly considered the decision to approach males, and all have involved species where females locate and choose males by their acoustic calls (e.g., Wiegmann 1999; Murphy and Gerhardt 2002; Baugh and Ryan 2010).

1.4 STUDY SYSTEM

Peafowl (genus *Pavo*; Figure 1.1) are large galliform birds with a long history as examples of sexual selection because of their remarkable plumage: the males possess a train ornament of over 200 elongated feathers, about 150 of which are tipped with a multi-coloured iridescent eyespot (Dakin and Montgomerie 2011; Figure 1.2). In developing his theory, Darwin (1871) frequently cites the peacock’s train, describing how the males erect and display this spectacular ornament to females during courtship. He even devotes a section of *The descent of man* to describing the eyespots of peacocks and related species as evidence for the gradual evolution of sexually selected traits.

During the breeding season, peacocks (the males) maintain territories in open areas called leks where they display their erect trains for females (Rands et al. 1984; Harikrishnan et al. 2010). Females (called peahens) visit these leks primarily for the purpose of courtship and copulation (Figure 1.3). Because these behaviours are easily observed on peafowl leks, the species is ideal for the study of mate choice. Moreover, because *Pavo cristatus* is one of a handful of bird species for which the visual system has been thoroughly characterized (Hart 2002), it is possible to estimate how females perceive male colour
signals during courtship. In the following sections, I describe previous work on the mating behaviour of peafowl, as well as studies of avian colour perception that provide the basis for the methods used in this thesis.

1.4.1 Mate choice in peafowl

Native to India, *Pavo cristatus* peafowl adapt readily to human-altered habitats, and they thrive in rural, parkland and suburban environments, in India and elsewhere. At present, there are hundreds of introduced populations of these iconic birds throughout the world. Peafowl in these introduced populations tend to be well habituated to the presence of humans, and as a result, there have been a number of studies of mate choice in semi-feral populations over the past twenty-five years. Some of the first were conducted by Marion Petrie, who began to study peafowl at the Whipsnade Zoological Park north of London, England, in the late 1980s. Based on observations of the mating activity of 10 males at one of the Whipsnade leks, Petrie et al. (1991) found a correlation between the number of eyespots displayed in a male’s train and his mating success, consistent with Darwin’s idea that mate choice led to the exaggeration of the train ornament. This was further supported by an experimental test in which Petrie and Halliday (1994) manipulated the trains of Whipsnade males and released them back into the population to prior to the start of the breeding season: removing a large number of a male’s eyespots effectively reduced his mating success.
**Figure 1.2** Eyespot iridescence. This sequence of photographs (a-f) shows a single eyespot feather rotating counter-clockwise, with the position of the light source and camera held constant. The light is positioned to the left of the camera. In the series (a-f), the feather rotates counter-clockwise from (a) an orientation facing towards the light (and to the left of the camera) to (f) an orientation facing slightly to the right of the camera. A full video of this can be seen at: [http://www.youtube.com/watch?v=KngAyPOTi4k](http://www.youtube.com/watch?v=KngAyPOTi4k)
Figure 1.3 (a) A peacock lek at the Assiniboine Park Zoo in Winnipeg, MB. Males use the same 2-3 m² display court, year after year. (b-g) A copulation. Males will continue to display their erect trains for a female after mating.
Subsequent work on the Whipsnade birds, as well as on populations in India and France, confirmed that the train ornament is a major focus of female choice: male mating success was found to be correlated with train length (Yasmin and Yahya 1996), the rate of male train displays (Loyau et al. 2005b), and the blue-green colour of the iridescent eyespots that tip the majority of the feathers in the male’s train (Loyau et al. 2007a). Studies of peafowl in Clères, France as well as three different populations in Canada and the US have also confirmed the original results of Petrie et al. (1991) that males with more eyespots achieve more copulations (Loyau et al. 2005b; Dakin and Montgomerie 2011) – although recent work has shown that this does not occur in populations where most males have close to the maximum number of eyespots (Takahashi et al. 2008; Dakin and Montgomerie 2011), suggesting that females cannot or do not discriminate subtle differences in eyespot number.

Because peafowl are amenable to captive breeding, they are also well suited to experimental approaches. Thus, there is evidence that when captive females are experimentally mated to preferred males, they lay more eggs (Petrie and Williams 1993), invest additional resources into those eggs (Loyau et al. 2007b), and bias the sex ratio of their offspring towards males (Pike and Petrie 2005). Other studies of captive-bred peafowl indicate that different features of the train ornament may be related to hematological (Møller and Petrie 2002) and genetic (Hale et al. 2009) measures of immunocompetence, suggesting that females may gain genetic benefits for their offspring as a result of their mate preferences for ornamental traits. This is also supported by a study of semi-feral birds in which a relation was found between train length and
circulating immune cells (Ros et al. 2009), and by a study demonstrating that males with more eyespots may be better able to cope with an experimentally-induced immune challenge (Loyau et al. 2005a). In addition, Petrie (1994) has found that when captive females are experimentally mated to preferred males, their offspring have increased growth rates and are more likely to survive, supporting the idea that females gain genetic benefit for their offspring as a result of mate choice – although in that 1994 study Petrie did not control for the fact that females may adjust their egg investment depending on the ornamental traits of their mate (Petrie and Williams 1993; Loyau et al. 2007b).

1.4.2 Vision in birds

Like all birds, peafowl have several different types of photoreceptor cells in their retinas: four types of single cone cells that are thought to be responsible for colour vision, one type of double cone, and one type of rod cell (Hart and Hunt 2007). Double cones, which are not found in mammals, are thought to be involved in detecting changes in luminance, texture or motion, although their function is not well understood (Cuthill 2006). Rod cells allow light detection in low light conditions, and are not sensitive to variation in colour.

Each of the different cone cell types contains a distinct visual pigment with a characteristic absorption spectrum, giving the retina the ability to detect changes in the spectral composition – or colour – of incoming light. Most mammals have two or three cone cell types; humans have three, in contrast with the four cone types possessed by birds.
Another difference between the visual systems of birds and mammals is that in birds, the cones are associated with pigmented oil droplets that filter the incoming light. This additional filtering may enhance the ability of the avian visual system to discriminate colours (Vorobyev et al. 1998). Indeed, birds are thought to perceive many colours that we humans cannot – the effective range of spectral sensitivity for avian cones extends well beyond ours into the UV range (Cuthill et al. 2000). Moreover, the extra cone type possessed by birds may provide them with an additional dimension of colour vision, relative to humans (Cuthill 2006). In order for this to be true, birds would have to possess neural mechanisms or “opponencies” for comparing the output of each of the four cone cell types with at least one other cell type; this could be achieved if they have at least three different opponent mechanisms involving all four cone types. Behavioural tests demonstrate that birds likely have the opponent mechanisms that would be sufficient for tetrachromatic colour vision (Osorio et al. 1999b; Goldsmith and Butler 2005), although the neural basis for these opponencies has not yet been characterized (Cuthill 2006).

1.4.3 Modeling colour perception

In 2002, Hart published a detailed description of the peafowl visual system, including the absorption characteristics of the retinal photoreceptor cells and oil droplets, as well as the spectral transmittance of the ocular media. These data can be used to determine the spectral sensitivity of each type of cone cell in the retina, allowing us to estimate the level of stimulation or photon catch of these cells as a function of the incoming light. Thus, for any coloured object or stimulus, given its reflectance spectrum, the irradiance spectrum of the ambient light, and the retinal cone sensitivities, we can calculate photon catch
values for the retinal cone cells. This allows us to convert stimulus reflectance spectra to measures that approach what a bird would perceive (Montgomerie 2006).

A variety of methods have been used to model avian colour perception based on photon catch, and all make different assumptions about the neural processes involved. In tetrahedral colour space models, photon catch values are used to derive coordinates for the stimulus colour in avian colour space (Goldsmith 1990), which can then be used to calculate variables describing what might represent bird-perceived hue and saturation, as well as the level of contrast birds would perceive between different colours (Endler 1990; Endler and Mielke 2005; Stoddard and Prum 2008). Colour contrast in these models is calculated as the Euclidean distance between two points in colour space. In his original colour space modeling procedure, Goldsmith (1990) assumed constant irradiance across all visible wavelengths of light. The more recent colour space developed by Endler and Mielke (2005) incorporates realistic ambient light spectra, as well as mathematical transformations to account for colour constancy (i.e., the tendency to perceive a given object as having the same colour under different light conditions).

In their analysis comparing the output of different colour space models, Stoddard and Prum (2008) reported that they found nearly the same results for plumage colour spectra from two bunting species with Goldsmith’s (1990) and Endler and Mielke’s (2005) procedures. This demonstrates that the transformations used to account for colour constancy in Endler and Mielke’s (2005) more recent model effectively counteract any effect of incorporating an ambient light spectrum. Furthermore, Stoddard and Prum
(2008) point out that other features of Endler and Mielke’s (2005) model, such as the logarithmic transformation of photon catch values, are based on aspects of colour vision that are not well understood in birds, so Goldsmith’s (1990) simpler model may be more accurate. In general, the application of these colour space models in birds is supported by behavioural evidence that zebra finch foraging preferences depend on the relative Euclidean distances between food items and the visual background (Maddocks et al. 2001).

Another method for modeling colour perception involves calculating the chromatic contrast of the stimulus against some other signal (typically the visual background) under the assumption that photoreceptor noise determines the threshold for further neural processing (Vorobyev and Osorio 1998). Predictions of these receptor noise-limited models accord well with sensitivities measured in behavioural experiments for a variety of di-, tri- and tetrachromatic organisms, including birds (Vorobyev and Osorio 1998).

An advantage of these receptor noise-limited models is that they are designed to allow prediction of an organism’s ability to discriminate pairs of colours (i.e., by estimating a threshold level for a “just noticeable difference” in units of the standard deviation of receptor noise; Vorobyev et al. 1998; Osorio et al. 2004). Threshold discrimination levels derived from these models accord well with experimental data on sensitivity and discrimination in the species where these psychophysical experiments have been performed (e.g., Maier 1992; Goldsmith and Butler 2005). Nevertheless, receptor noise-limited models have been used to estimate birds’ ability to discriminate colours in a
number of studies, establishing that tetrachromacy and oil droplets may enhance birds’ ability to discriminate plumage colours, and testing how different illumination conditions affect colour discrimination and constancy (Vorobyev et al. 1998). These models have also been used to demonstrate sexual dimorphism in many bird species that appear monomorphic to human observers (Eaton 2005), and to design stimuli for behavioural tests of colour discrimination and memory in birds (Osorio et al. 1999a).

A third method for modeling colour perception involves estimating the neural signal resulting from opponent mechanisms as a ratio of different cone cell photon catch levels (e.g., Evans et al. 2010; Spottiswoode and Stevens 2011) – although as noted previously, these neural opponencies have not been well characterized in birds, and must therefore be assumed (but see Osorio et al. 1999b). Moreover, it is not clear that the mathematical computations used to compare photon catch levels in these models necessarily correspond to the way these neural signals are combined.

1.4.4 Colour perception and peafowl

These models of how animals perceive colour can help us better understand how they make decisions, from foraging (e.g., Maddocks et al. 2001) to mating (e.g., Loyau et al. 2007a) and other social choices. They are leading to new insights into the selective pressures shaping the evolution of colour vision (e.g., Osorio et al. 2004) and animal colouration (e.g., Hästad et al. 2005; Macedonia et al. 2009; Kemp et al. 2009; Maan and Cummings 2012), challenging our assumptions (e.g., Delhey et al. 2010), and drawing our attention to new questions, such as why some colour patterns have not yet evolved
(e.g., Stoddard and Prum 2011). The peacock is one of a relatively short list of birds for which cone cell sensitivities are readily available (Hart 2002; Hart and Hunt 2007), and their sensitivity spectra have been applied in several of these recent studies. For instance, peafowl have been used as a stand-in for raptor predators living in Sweden (Håstad et al. 2005) and Mexico (Macedonia et al. 2009) in studies examining how different prey organisms would appear to avian predators.

Loyau et al. (2007a) applied a receptor noise-limited model of peafowl colour vision in their study of female choice for the peacock’s iridescent plumage colour. To do this, they measured reflectance spectra of the blue-green portion of the eyespot with the measurement probe held at various angles relative to the feather, which allowed them to capture iridescence, or the change in colour with viewing angle (Figure 1.2). Loyau et al. (2007a) then modeled how females would perceive the chromatic contrast of the blue-green plumage against a constant background of green vegetation. When comparing these data with observations of male mating success, Loyau et al. (2007a) found that both the brightness of the blue-green patch and perceived iridescence – or the change in chromatic contrast against the background with viewing angle – were correlated with mating success, suggesting that females may prefer certain males based on their colour traits.

More recently, I found that peacocks orient their erect trains at an angle of about 45° to the right of the sun’s azimuth, on average, during their train-rattling courtship displays (Dakin and Montgomerie 2009). During this display – which always precedes copulation – males rapidly vibrate their erect train feathers in front of a female (see Figure 1.4 for
images and high-speed video). In that earlier study, I also showed that males do not orient their trains relative to the sun when they are performing the wing-shaking display that typically precedes bouts of train-rattling. At the same time, I showed using experiments with a stationary model female that males adjust their display behaviour according the position of the female relative to the sun’s azimuth (Dakin and Montgomerie 2009).

When females are positioned on the shaded side of the train, males tend to perform the wing-shaking display first – often walking backwards towards the target female before they begin to vibrate their trains. In contrast, when females are positioned on the sunny side of the train, males often begin with the train-rattling display. Overall, these results suggest that males adjust their behaviour to ensure that females will view them at certain light angles, supporting Loyau et al.’s (2007a) discovery that iridescence is an important component of courtship in this species.

1.5 GOALS

In this thesis, I attempt to build on earlier studies of mate choice in peafowl by considering female colour vision and male display behaviour during courtship. I begin by applying tetrahedral colour space models to measurements of all three of the main colour patches in the eyespot pattern, taken at light angles similar to those males use during display. My goal in Chapter 2 is to ask how these colours influence male mating success across three different study populations.

I then ask how females use male colour traits and other features of the train ornament when deciding to visit different males for courtship (Chapter 3). I look for evidence of
memory during natural courtship interactions in which females often visit different males over a period of several days.

In Chapter 4, I ask how sensory biology and light conditions might have shaped the peacock’s eyespot colours and display behaviours. I begin by comparing the plumage colours of two closely-related peafowl species, *Pavo cristatus* and *P. muticus*, and I examine how females would perceive them under different light conditions. I also investigate how typical *P. cristatus* male courtship behaviours affect how females perceive the eyespot colours – which in turn might affect female choice. Together, these studies demonstrate that peafowl are a promising system for further investigation of the mechanisms of mate choice, and I end by discussing the potential for future study (Chapter 5).
Figure 1.4 (a) A peacock vibrates his iridescent train during the train-rattling display. (b) The same male viewed from behind. The vibration is caused by the motion of the erect tail feathers, held vertically to support the erect train. High-speed video of this display can be seen at: http://www.youtube.com/watch?v=iHCU1Vcifkg
1.6 REFERENCES


Darwin C. 1838. Correspondence Project Database, Darwin’s notes on marriage. See http://www.darwinproject.ac.uk/darwins-notes-on-marriage#_edn1


CHAPTER 2. How iridescent plumage ocelli influence peacock mating success

2.1 ABSTRACT

Each of the multi-coloured eyespots (ocelli) on the peacock’s (*Pavo cristatus*) train is a complex structure with a purple-black center surrounded by concentric blue-green and bronze-gold regions. To investigate the influence of all three of these colours on male mating success, we used a physiological model of peafowl vision to quantify those colours as females would perceive them during male courtship displays. Males display at 45° to the right of the sun’s azimuth (on average) with the female directly in front, so we investigated how colors would be perceived when illuminated at 30°, 45° and 60° to the right of a female observer. We studied 34 males displaying at leks in three feral populations and quantified their copulation success and the colours of their eyespots. Eyespot colouration explained half of the observed variation in peacock mating success, with the hue and iridescence of the blue-green patch being the most important colour variables. When we experimentally masked ocelli on 9 males, their copulation success declined almost to zero, confirming that the eyespots are a major focus of female attention and not a trait correlated with something else that influences female choice. Thus our study shows that the blue-green eyespot colour overwhelmingly influences peacock mating success. The influence of the other eyespot colours on male success is minimal at best, raising questions about their function.
2.2 INTRODUCTION

For more than 20 years, research on sexual selection in peafowl has focused mainly on the number and distribution of eyespots in the peacock’s train as the target of female choice (Manning and Hartley 1991; Petrie et al. 1991; Petrie and Halliday 1994; Møller and Petrie 2002). Recent evidence, however, suggests that these traits may not have much influence on male reproductive success (Takahashi et al. 2008; Dakin and Montgomerie 2011), and that the colour of the eyespots may instead be important (Loyau et al. 2007). We designed the present study to quantify the colours of the eyespots on courting males, to estimate how females might perceive those colours, and to determine whether natural variation in the eyespots’ colours had any influence on male reproductive success.

Peacocks have an elaborate ornamental train that includes more than 150 eyespot feathers (see Table 2 of Dakin and Montgomerie 2011), each with an iridescent eye-like pattern near the feather tip, called an eyespot or ocellus (Figure 2.1). These ocelli have a dark purple-black center surrounded by two large concentric regions of blue-green and bronze-gold, and a few narrower outer bands of additional colours. All three of the main eyespot colours are iridescent, produced by highly organized nanostructures of melanin rods connected by keratin within each barbule comprising the ocellus (Zi et al. 2003). The different eyespot colours are the result of variations in two parameters describing these crystal-like nanostructures—the lattice constant that defines the spacing between the melanin rods in the nanostructure, and the total number of layers of rods (Zi et al. 2003).
The peacock’s eyespots have fascinated scientists for centuries because of their sheer complexity. They were, for example, included in Isaac Newton’s earliest studies of structural colouration in nature (Newton 1704). Moreover, their “trifling particulars of structure” evidently made Charles Darwin sick with worry (Darwin 1860) long before he proposed his theory of sexual selection to explain the evolution of ornamental traits by female preferences (Darwin 1871). In support of Darwin’s idea that the beautiful eyespots are the product of sexual selection, Loyau et al. (2007) showed that both the brightness of the large blue-green portion of the eyespot, as well as its iridescence, were positively correlated with mating success in a feral peafowl population in France. That study looked only at the blue-green colour of the eyespot, possibly because it is the region with the greatest spectral purity (Figure 2.2), and thus is the most striking color to human eyes.

In the present study we measured all three eyespot colours on a sample of courting peacocks, and used visual modeling to estimate how peahens would see those eyespots during courtship displays. We recorded the copulation success of those same males during the spring breeding season. Upon finding a correlation between male mating success and eyespot colours, we conducted a manipulative experiment to determine whether the eyespot itself influenced female choice, rather than some other character simply correlated with the eyespot colours that we measured. We have previously shown that peacocks display their erect trains so that the target female is directly in front and the train itself is oriented at 45° to the right of the sun’s azimuth (Dakin and Montgomerie 2009). We therefore measured eyespot colours illuminated at 30°, 45° and 60° to the right
of the vertical feather surface so that our measurements would more closely mimic the way these feathers would be seen by females during courtship.

2.3 METHODS

2.3.1 Field methods

We studied peafowl in three feral populations: (i) Assiniboine Park Zoo (APZ) in Winnipeg, MB, Canada (April-May 2007), where about 60 peafowl were free-ranging over 50 ha of pens and woodland; (ii) Toronto Zoo (TZ) in Toronto, ON, Canada (April-June 2007), where about 30 peafowl were free ranging over 250 ha of pens and woodland; and (iii) Los Angeles Arboretum (LAA) in Arcadia, CA, USA (February-April 2008-2010), where >100 peafowl lived in 50 ha of parklands and the surrounding residential areas. The LAA birds were feral year-round, whereas the APZ and TZ populations were housed in large indoor pens during the coldest winter months (December-March). All three populations were wild-type birds that mated on leks, similar to populations in India (Hillgarth 1984; Harikrishnan et al. 2010).

We caught birds prior to the start of the breeding season (April at APZ and TZ; January-March at LAA), and marked them with numbered leg bands. To measure colours, we removed 5 eyespots from the train of each captured adult male by cutting the rachis immediately below the eyespot on 5 of the longest major eyespot feathers. For a separate study of train morphology (Dakin and Montgomerie 2011), we also removed one of the shortest major eyespot feathers from each male in LAA (n = 11) in 2010, as well as 15-20 of the eyespots from the longest major eyespot feathers from 6 males at APZ and 1 male
at TZ in 2007. Feathers were stored in opaque paper envelopes prior to taking colour measurements.

Several previous studies have shown that the number of eyespots displayed in the peacock’s train ornament can influence female mate choice (Petrie et al. 1991; Petrie and Halliday 1994; Loyau et al. 2005; Dakin and Montgomerie 2011), and that this relation can be independent of an effect of eyespot colour (Loyau et al. 2007). For this reason, we counted the number of eyespots displayed by each male, so that we could account for eyespot number in our statistical analyses. To quantify the number of eyespots displayed in each male’s train during the breeding season, we digitally photographed displaying males after any eyespots had been removed for analysis (see Dakin and Montgomerie 2011 for details).

Møller and Petrie (2002) indicated that the size of individual eyespots may be related to male immunocompetence. Based on this, one might predict that eyespot size could potentially influence mate choice, so we also measured the total area of each of the five eyespots removed from each male, and calculated an average area for use in further analyses. Eyespot area was determined from digital images taken on a flatbed scanner (hp Scanjet 7400c) of each eyespot laid flat against a standard grey card background. We used Adobe Photoshop 10.0.1 (Adobe Systems 2008) to outline the outer edge of the bronze region of each eyespot, and converted the area of this shape to cm² using a ruled scale on the grey card background.
FIGURE 2.1 Directional reflectance spectrometry of a peacock eyespot feather using illumination angles of 30°, 45° and 60° from the female’s viewing position during courtship, with measurements taken at 0°. All feathers were measured on the right side when viewed from the front, as shown. The region of measurement for each of the purple-black (PB), bronze-gold (BZ) and blue-green (BG) colour patches is marked with a dot.
FIGURE 2.2 Typical reflectance spectra for peacock eyespot colours; curves are averages of measurements taken from a single feather from each of 10 males. Coloured lines indicate spectra for the purple-black (PB), blue-green (BG) and bronze-gold (BZ) patches at illumination angles of 30°, 45° and 60° from the female’s viewing position during courtship.
2.3.2 Quantifying peacock mating success

We used slightly different methods each year to observe adult males attending leks and to record their copulation success (APZ: 14 males; TZ: 5 males; LAA 2008: 13 males; LAA 2009: 16 males; LAA 2010: 11 males). In 2007 (at APZ and TZ) and 2008 (at LAA), we conducted focal watches of 1-5 males at different lek sites for periods ranging from 0.5-2.5 h during peak lekking periods (07:00-12:00 and 16:00-18:00 local times; Petrie et al., 1991; R. Dakin, unpublished data). We recorded the number of successful copulations obtained by each focal male (APZ: 34 copulations among 14 males, during 80 h of observation; TZ: 6 copulations among 5 males, 115 h; LAA 2008: 25 copulations among 13 males, 160 h). In 2010 (at LAA), we used the same methods, but performed focal watches of males on 4 leks continuously (08:00-18:00 local time) for a 13-day period (15-27 Mar), recording all copulation events (19 copulations among 11 males during 506 h of observation). In 2009 (at LAA), however, we tracked focal females (8 marked and 20 unmarked but individually identifiable birds) as they visited males on 6 leks during peak lekking times, recording all copulations observed (23 copulations among 16 males).

We tracked females for periods of 54 min on average (95% CI [47-61], range 10-205, n = 132 observation periods on 28 females), for a total observation time of 121 h.

These observations provide accurate measures of a male’s mating success relative to his competitors in any given year, but not necessarily between years because we varied our methods of observation. Thus, to compare males across populations and years studied, we used the number of copulations obtained by each male standardized within each
population-year sample (mean = 0 and SD = 1). In total we recorded the mating success of 36 males (17 at LAA, 5 at TZ, 14 at APZ). Birds at APZ and TZ were studied in only one year (2007) but individual males at LAA were studied in one (n = 6), two (n = 8) or three (n = 3) years, with eyespots being removed and measured from those males in each year that they were studied.

2.3.3 Measuring colours and iridescence

For each male, we selected for colour measurement and analysis the eyespot with the most symmetrical purple-black patch of those collected from him in each year that he was observed. See Appendix A for further details on our choice of feathers to measure.

We measured the iridescent colours of the three largest patches (purple-black, blue-green, and bronze-gold) on the right side of every eyespot, as viewed from the front (Figure 2.1) and quantified these colours across the bird-visible spectrum (300-700 nm). To do that, we used a USB4000-UV-VIS spectrometer (Ocean Optics, Dunedin, FL, USA) and took measurements at different angles of incident light by mounting illumination and measurement probes in a goniometer. We mounted collimating lenses onto the ends of 400 µm optical fibers for both illumination (560 mm from the sample surface) and measurement (470 mm from the sample) of a spot about 2 mm in diameter on the feather’s surface. The alignment of the two beams was confirmed by shining a laser pointer’s beam down the measurement fiber. We measured reflectance at 90° to the feather surface (i.e., normal), since peahens are positioned directly in front of males during their pre-copulatory train-rattling displays (Dakin and Montgomerie 2009). For
illumination, we used an Ocean Optics DH-2000 Deuterium Tungsten Halogen light source (output 215-2000 nm), with the illumination probe set at angles 30°, 45° and 60° to the right of the measurement probe (Figure 2.1), equivalent to a male displaying at those angles to the right of the sun’s azimuth. We chose those angles to span the normal range of illumination angles during the male’s display, not to determine which angle of illumination best predicted male success. All measurements were taken in a darkroom to eliminate any effects of ambient light.

Reflectance was taken relative to a white standard made of Teflon™ tape layered to be the same thickness as the eyespot feathers, to ensure that it could be mounted in our apparatus at the same distance from the reflectance and illumination probes as the feather surface. Dark standard readings were taken in a small black chamber that eliminated reflected light. We took the average of 10 scans at 100 ms integration time, with a boxcar smoothing function of 12 pixels, using SpectraSuite 2.0 software (Ocean Optics 2009). Every 15 minutes we recalibrated dark and white standard readings, to reduce the effects of instrument drift. We measured each of the three colour patches on every feather twice—re-mounting the samples in the apparatus between measurements—and used the average spectrum from the two measurements of each colour patch. We repeated this procedure for the three illumination angles (30°, 45° and 60°), keeping the illumination probe locked in position for all feather measurements at a given viewing geometry to reduce measurement error.
2.3.4 Vision models

We used the measured reflectance spectra in conjunction with models of peacock vision to quantify how females might perceive the eyespot colours. There are different approaches to modeling avian vision (e.g., Endler and Mielke 2005; Stoddard and Prum 2008; Spottiswoode and Stevens 2010) that make different assumptions about the factors that affect what colours birds actually perceive. Recognizing that there is no consensus about which type of vision modeling is most accurate, we analyzed our data using a tetrahedral colour space model (Goldsmith 1990; Endler and Mielke 2005; Stoddard and Prum 2008) of avian vision, assuming that illumination was constant across all bird-visible wavelengths (300-700 nm).

We based the model on the peafowl retinal cone sensitivities reported in Hart (2002: Figure 7), each normalized to a total area of 1.0 under the spectral sensitivity curve in the bird-visible region (following Stoddard and Prum 2008). We converted each reflectance spectrum from a colour patch to a locus defined by three spherical coordinates representing chroma ($r$) and hue ($\phi$ and $\theta$) within the tetrahedral colour space (Supplementary Material Figure S1). The values for hue ($\phi$ and $\theta$) are both angles measured from the achromatic origin. $\phi$ is the vertical angle (range from $+90$ to $-90^\circ$), or hue latitude, and represents the UV-violet contribution to perceived colour with more positive values indicating more UV perceived. $\theta$ is the angular displacement ($+180^\circ$ to $-180^\circ$) around a circle parallel to the base of the tetrahedron, or hue longitude, where perceived red-greens (e.g., bronze) are close to $0^\circ$, reds and purples are negative, greens and blues are positive, and blue-greens have high positive and negative angles. Following
Stoddard and Prum (2008), we also calculated achieved chroma, which is an estimate of the ratio of \( r \) to the maximum possible chroma along the hue vector (defined by \( \phi \) and \( \theta \)) for that locus.

Using this tetrahedral color space model, we calculated three sets of colour variables to describe what a female could see when viewing the eyespot. First we calculated the iridescence of each patch, as the Euclidean distance in tetrahedral avian colour space (‘color span’ from Stoddard and Prum 2008, or ‘\( \Delta r \)’ from Endler and Mielke 2005) between the colour loci for the reflectance spectra of each patch at 30° vs 60°, 30° vs 45° and 45° vs 60° illumination angles. These variables provide an estimate of the perceived difference in colour when the feather is illuminated at those pairs of angles (Figure 1). Our results (see below) suggested that the colour span between 60° vs 30° was the best measure of iridescence for the blue-green patch, so we used that span as an index of iridescence for all three colour patches.

Second, we calculated the chromatic contrasts between adjacent colour patches (blue-green vs. purple-black; blue-green vs. bronze-gold) as the colour spans (as defined above) between those patches at each illumination angle (30°, 45°, 60°). We assumed that each colour is seen by the female in relation to the adjacent colour patch, and not against a background of vegetation as quantified by Loyau et al. (2007). Third, we determined both hue (\( \phi \) and \( \theta \)) and achieved chroma of each patch at each angle of illumination (see Stoddard and Prum 2008 for details of calculations).
Thus, from the average reflectance spectrum from each colour patch on each eyespot, we calculated (i) a measure of iridescence (a dynamic colour variable), as well as (ii) two colour contrasts at each illumination angle and (iii) three colour variables (hue \( \rho \), hue \( \theta \) and achieved chroma) for each colour patch at each illumination angle, for a total of 36 colour variables.

2.3.5 Manipulating eyespot colour

We tested whether eyespot colour itself influenced male mating success in 2008 by covering the central purple-black and iridescent blue-green regions (but not the bronze-gold patch) of each eyespot in the trains of 9 males in the LAA population with a waterproof sticker (insignia repair tape, North Sails), cut in the shape of the blue-green region (Figure 2.1). This adhesive-backed polyester material is light (about 127 g/m\(^2\)) and the total mass of material applied to each male as was <11 g (estimated from 0.067 g per sticker, 150-170 stickers per male). We applied stickers to the front side of all eyespots on 9 males: black-coloured stickers on 5 males and white-coloured stickers on 4 males (Figure 2.3). It should be noted that this treatment altered male appearance well beyond the normal range of variation among males (Figure 2.3). Control males \( n = 4 \) were handled similarly, but no stickers were applied.

We chose males for these three different treatments haphazardly by alternating treatment type as we caught birds, since we could not be certain of the total number of males we could catch before lekking began. The sticker material is waterproof with a long-lasting adhesive that males could not easily remove by preening, and nearly all stickers remained
on the eyespots for the duration of the breeding season (>2 months), with treatment males displaying an average of only 4.3 eyespots (range 1-7) without stickers when we photographed them during the breeding season about a month after the stickers were applied.

To assess the effects of the colour manipulation on mating success, we conducted focal watches of 1-4 males at a time at 7 different lek sites during peak lekking periods as described above. For each male, we recorded (i) the durations of attendance on the lek, train displays (i.e., train erect), and bouts of preening behaviour, and (ii) the numbers of “train-rattling” bouts (see Dakin and Montgomerie 2009), copulation attempts (“hoot-dashes”; Petrie et al. 1991), and successful copulations.
FIGURE 2.3 Experimental manipulation of peacock eyespot colours. Males with (a) black and (b) white stickers masking the purple-black and blue-green patches on the eyespot feathers in the train ornament.
As an additional measure of female interest, we quantified female visitation rate to each male in this experiment as the number of 5-min intervals where he had at least one female visitor present divided by the number of 5-min intervals that the male was observed on the lek. We defined a visitor as any female <5 m from the focal male when his train was erect, and not closer to any other adult male (see also Dakin and Montgomerie 2011).

We attempted to distribute focal watches equally among the different lek sites. The mean total time that each male was in view was 12.0 h (95%CI [9.6, 14.4], range 6.1-18.9, n = 13 males), with the variation in total time observed was due to natural variation in male attendance and not because of a biased distribution of observation periods. To account for different males being observed for different periods, we calculated male-specific rates of train-rattling bouts, copulation attempts, and successful copulations per hour. As a measure of display rate, we divided the total time males spent with their trains erect by the total time observed on the lek; male preening rate was calculated the same way. We used rates of train-rattling bouts, female visitation, copulation attempts and successful copulations as measures of male mating success (Dakin and Montgomerie 2011). Since the presence of a female usually caused males to raise their trains, display rate and female visitation rate are highly correlated (r = 0.64, P = 0.02, n = 13 males). Thus, we defined a male’s tendency to display independent of female visitation as ‘residual display rate’, calculated as the residuals of male display rate regressed on female visitation rate. We use effect sizes to assess the biological importance of differences between experimental treatments and the control.
Since the number of males in each treatment for this experiment was necessarily small, we use effect sizes to assess the biological importance of differences between experimental treatments and the control.

2.3.6 Ethical note

All methods used in this study were approved by the Queen’s University Animal Care Committee (Animal Utilization Protocols Montgomerie-2005-044-Or and Montgomerie-2009-006-Or), and the animal care committees of the Assiniboine Park Zoo, Toronto Zoo and Los Angeles Arboretum. The handling procedures and manipulations of eyespot colours did not result in injury to any animals.

2.3.7 Analyzing data

We used JMP 10.0.1 and R 2.15.2 (R Development Core Team 2011) for all statistical analyses. Several variables describing male mating success (standardized copulation number in the pooled population-year sample, and both copulation rate and copulation attempt rate in the colour manipulation experiment) were zero-inflated so we used fourth-root transformations to normalize residuals (Quinn and Keough 2002). For the colour manipulation experiment, we used parametric Dunnett’s tests to compare experimental treatments (with black or white stickers) to control males (no stickers).

We checked the distribution of each tetrahedral colour space variable to ensure that it was unimodal and normally distributed. Because theta is measured as the angle around a circle, we wanted to ensure that the distributions of theta did not include 180° as that
would result in bimodal pattern. Only the blue-green $\theta$ values were clustered around $\pm 180^\circ$ so we added 360$^\circ$ to negative values to normalize those distributions.

To assess collinearity between predictor colour variables, correlations between colour variables were calculated by resampling because several males were measured in different years. To do this we chose one value per male at each of 1000 iterations and calculated the mean of $r$ and $P$ over all iterations. Preliminary analysis of the correlations among colour variables revealed that two males that we studied at APZ were serious outliers (see Supplementary Material Figure S2). Neither of these males obtained any copulations, so to ensure that they would not bias our results, we removed them from further analyses that we report here. Including these males in the analyses resulted in the same general conclusions, in most cases resulting in stronger relations than the ones we report.

To explore whether our measurements of the eyespot colours might explain variation in male mating success, we constructed general linear mixed models to predict (fourth-root transformed) standardized copulation number in our pooled population-year sample using an information-theoretic (IT) framework (Burnham et al. 2011). This IT approach has the virtue of revealing different models that are all reasonable fits to the data, and thus avoids the problem of eliminating potentially important variables as a result of collinearity. Because there were often many potential predictor variables in a given analysis, and because some of these variables were highly correlated with one another, we took an exploratory approach to model building (Zuur et al. 2009). First, we built a model from
the blue-green iridescence predictors as iridescence of this patch has previously been shown to correlate with male mating success in this species (Loyau et al. 2007). Second, we built a model using only the colour contrast variables. Third, we built separate models for each color patch using the hue (\(\phi\) and \(\theta\)) and achieved chroma from the tetrahedral colour space model for that patch. Fourth, we built a global model to predict male copulation success using the predictors included in the best model from each set.

Models in each set with \(\Delta AICc \leq 2\) were considered to be equally likely ‘top models’, given the data (Burnham et al. 2011). We report the averaged model from the top models in each set (see Appendix A for a summary of all of the top models), and a coefficient of determination (pseudo-R\(^2\), based on the likelihood ratio test) that represents the proportion of variance in the response that is explained by the predictors in each model. We used an adjusted-pseudo-R\(^2\) scaled to the maximum possible R\(^2\) for each model (Nagelkerke 1991).

We used the lme function in the R package nlme (v3.1-103) and the dredge function in MuMIn (v1.7.7) to compare and evaluate models in each set, to do model averaging and to calculate the adjusted-pseudo-R\(^2\) values. Since some males at LAA were studied in more than one year, we assigned male identity as a random variable. We also included the number of eyespots displayed on a male’s train as a predictor in all models. Although the number of eyespots does not appear to influence male copulation success across the normal range of variation in our study populations, some males in our sample had >20
eyespot feathers removed experimentally, enough to reduce their mating success (Dakin and Montgomerie 2011).

All predictor variables were standardized before analysis so that the partial regression coefficients could be used to assess the relative strength of each predictor. The relative importance of each predictor is also determined during model averaging as the sum of the Akaike weights (up to a maximum of 1.0) from all of the top models in which that predictor appears.

2.4 RESULTS

2.4.1 Number and size of eyespots

The males we studied had from 127 to 162 eyespots (mean = 148, 95% CI [145, 151], n = 48 samples from 34 males). The eyespots we removed ranged in size from 8.3 to 14.9 cm$^2$ (mean area = 11.1 cm$^2$ [10.7, 11.6]. Neither the number (t = 1.12, P = 0.29) nor the average size (t = 0.72, P = 0.49) of the eyespots a male displayed had a significant effect on male copulation success, in a model that included both as predictors. However, since all 16 of the males in our study displaying fewer than 140 eyespots obtained no copulations (as expected, see Dakin and Montgomerie 2011), we included eyespot number as a potential predictor in all subsequent models. Eyespot number alone explained only 2% of the variation in male copulation success (r = 0.15, P = 0.41, n = 48 samples from 34 males).
2.4.2 Eyespot colours

Figure 2.2 shows typical reflectance spectra for each of the three large colour patches in the eyespot, at illumination angles of 30°, 45° and 60° relative to the female viewer (see also Figure 2.1). Correlations among blue-green iridescence variables are all significant, both positive and negative. Correlations among the six colour contrast variables are all positive and almost all were significant. Correlations among the tetrahedral colour space variables, achieved r, phi and theta, are both positive and negative, with about two-thirds of the pairwise correlations being statistically significant. This level of collinearity can make regression parameters unreliable, but should not unduly influence the predictive power of the resulting model (Quinn and Keough 2002:127). Because of this collinearity, however, we are cautious about interpreting the relative importance of specific colour variables in predicting male copulation success (see Discussion).

2.4.3 Iridescence and mating success

For the blue-green patch, the only measure of iridescence included in the top models was the difference in colours illuminated at 60° versus 30° (i.e. iridescence 60°/30°; Table 2.1). This is not surprising since this measure of iridescence represents the largest difference in reflected colours, compared to iridescences 60°/45° and 45°/30°, which are both also positively and significantly correlated with iridescence 60°/30° (r > 0.48, P <0.01, n = 48 samples from 34 males). Note, however, that iridescence 60°/45° is negatively, and not significantly, correlated with iridescence 45°/30° in the blue green patch (r = –0.28, P = 0.17, n = 48 samples from 34 males). Based on these results, we
used only iridescence 60°/30° in subsequent analyses of iridescence for all three colour patches.

Iridescence 60°/30° of both the blue-green and bronze-gold patches were included in the top models (Table 2.1) in the iridescence set, controlling for the number of eyespots, and the averaged model explained 19% of the variation in male copulation success. Interestingly, the iridescence of the bronze-gold patch is a negative predictor in this model, suggesting that females prefer males with less iridescence in the bronze-gold patch. The iridescences of these two patches are not significantly correlated (r = 0.16, P = 0.38, n = 48 samples from 34 males).
TABLE 2.1 Models to predict male copulation success from each set of colour variables, and all of these variables combined (see text for details of model building). For each set the average of the top models is presented, with the variables included in the best model in each set highlighted in bold. Also shown is the adjusted likelihood-ratio-based pseudo-$R^2$, a measure of the proportion of variation in male copulation success explained by the best model. See Table A1 in Appendix A for a summary of the top models ($\Delta$AICc ≤ 2) in each set.

<table>
<thead>
<tr>
<th>model set</th>
<th>predictors in model</th>
<th>Std coefficient [95%CI]</th>
<th>Relative importance</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue-green iridescence</td>
<td>no. of eyespots</td>
<td>0.22 [−0.12, 0.56]</td>
<td>0.52</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>blue-green iridescence $60^\circ/30^\circ$</td>
<td>0.37 [0.07, 0.67]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iridescence at $60^\circ/30^\circ$ of all colour patches</td>
<td>no. of eyespots</td>
<td>0.22 [−0.11, 0.55]</td>
<td>0.55</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>blue-green iridescence $60^\circ/30^\circ$</td>
<td>0.38 [0.08, 0.68]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>bronze-gold iridescence $60^\circ/30^\circ$</td>
<td>−0.22 [−0.52, 0.08]</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Colour contrasts</td>
<td>blue-green x bronze-gold at $30^\circ$</td>
<td>0.27 [−0.11, 0.64]</td>
<td>0.51</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>blue-green x bronze-gold at $60^\circ$</td>
<td>0.20 [−0.13, 0.54]</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blue-green x bronze-gold at $45^\circ$</td>
<td>0.19 [−0.15, 0.53]</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blue-green x purple-black at $30^\circ$</td>
<td>−0.13 [−0.57, 0.32]</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>no. of eyespots</td>
<td>0.11 [−0.23, 0.45]</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Purple-black patch</td>
<td>achieved chroma at $60^\circ$</td>
<td>−0.25 [−0.64, 0.14]</td>
<td>0.59</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>phi at $60^\circ$</td>
<td>−0.29 [−0.67, 0.09]</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>no. of eyespots</td>
<td>0.16 [−0.19, 0.52]</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Blue-green patch</td>
<td>no. of eyespots</td>
<td>0.22 [−0.08, 0.51]</td>
<td>0.67</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>$\theta$ at $30^\circ$</td>
<td>2.95 [1.73, 4.16]</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\theta$ at $45^\circ$</td>
<td>−0.45 [−1.40, 0.50]</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\theta$ at $60^\circ$</td>
<td>−2.50 [−3.92, −1.07]</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phi at $30^\circ$</td>
<td>−2.05 [−3.23, −0.87]</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phi at $45^\circ$</td>
<td>−0.48 [−1.25, 0.30]</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phi at $60^\circ$</td>
<td>1.91 [0.54, 3.27]</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Bronze-gold patch</td>
<td>no. of eyespots</td>
<td>0.21 [−0.13, 0.54]</td>
<td>0.27</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>achieved chroma at $45^\circ$</td>
<td>0.25 [−0.08, 0.58]</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>achieved chroma at $60^\circ$</td>
<td>0.17 [−0.12, 0.46]</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\theta$ at $45^\circ$</td>
<td>−0.17 [−0.53, 0.18]</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\theta$ at $60^\circ$</td>
<td>−0.19 [−0.53, 0.15]</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phi at $30^\circ$</td>
<td>0.19 [−0.15, 0.54]</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phi at $45^\circ$</td>
<td>0.15 [−0.18, 0.47]</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phi at $60^\circ$</td>
<td>0.21 [−0.12, 0.54]</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>All variables</td>
<td>no. of eyespots</td>
<td>0.22 [−0.07, 0.51]</td>
<td>0.69</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>bronze-gold achieved chroma at $45^\circ$</td>
<td>0.13 [−0.14, 0.40]</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blue-green $\theta$ at $30^\circ$</td>
<td>−2.51 [−3.89, −1.12]</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blue-green $\theta$ at $60^\circ$</td>
<td>−1.94 [−3.11, −0.77]</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blue-green phi at $30^\circ$</td>
<td>1.74 [0.50, 2.98]</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>
2.4.4 Colour contrasts and mating success

The averaged model contained four colour contrast variables but explained only 5% of the variation in male copulation success (Table 2.1). Only the contrast between the blue-green and bronze-gold patches illuminated at 30° was included in the best model in this set (Table A1 in Appendix A), and had by far the highest relative importance in the averaged model (Table 2.1). All four colour contrast variables were highly correlated with one another (r > 0.74, P <0.0001, n = 48 samples from 34 males).

2.4.5 Different eyespot patch colours and mating success

All of the top models based on tetrahedral colour space variables for the blue-green patch included (i) hue (theta) with the patch illuminated at both 30° and 60°, and (ii) hue (phi) with the patch illuminated at 30° (Tables 2.1 and A1). The averaged model also included eyespot number (Table 2.1), and the best model explained 51% of the variation in male copulation success.

The best model for the purple-black patch was the null model (Table A1), but some of the top models also included eyespot number, as well as hue (phi) and achieved chroma with the patch illuminated at 60°, and these are all included in the averaged model (Table 2.1).

The best model for the bronze-gold patch included only achieved chroma with the patch illuminated at 45°, and explained 11% of the variation in male success. Some top models included one or more of the other bronze-gold colour variables (Table A1) but all of these had low relative importance in the averaged model (Table 2.1).
For the blue-green and bronze-gold patches, hue \((\theta)\) values for feathers illuminated at 60° and 30° were very highly correlated with iridescence 60°/30° \((R^2 > 0.95\) in each case). Thus the iridescence of these two patches can be described as the difference in hue captured by the LW, MW, and SW cones, and not the UV cones. Because of the strong relation between iridescence and hues \((\theta)\) of feathers illuminated at 30° and 60°, we did not include iridescence measures as predictors when constructing the combined model below.

### 2.4.6 Global model of male mating success

To assess the influence of iridescence, contrasts and colour variables together we constructed a model using all of the variables in the best models for colour contrasts, and tetrahedral colour space variables from each patch as potential predictors (see Table A1). As noted above, we chose to include tetrahedral colour space variables for hue \((\theta)\) at 60° and 30°, rather than iridescence variables, as these were highly correlated. The top models from this analysis (AICc ≤2) included all of the variables in the best models from the other model sets, except the hue \((\theta)\) of the bronze-gold patch illuminated at 60° (Table 1). The best model in this ‘all variables’ set included only colour variables from the blue-green patch and the number of eyespots, and explains 51% of the variation in male success (Table 2.1).

Thus, males had higher copulation success if they had (i) more eyespots with (ii) more iridescence in the blue-green patch (i.e. greater difference in \(\theta\) for this patch when
illuminated at 30° vs 60°), (iii) a blue-green hue more toward the blue (negative \( \phi \)) when illuminated at 30° while more toward the UV (positive \( \phi \)) when illuminated at 60°, and (iv) a more saturated bronze-gold patch (higher achieved chroma) illuminated at 45° (see ‘all variables’ model set in Table 2.1). Note that the distribution of data in Figure 2.4 shows that the relations between copulation success and the color predictors in the averaged model (Table 2.1) are very similar among our three study populations, with no obvious outliers.

2.4.7 Colour manipulation experiment

The train length (Figure 2.5a) and number of eyespots displayed (Figure 2.5b) did not differ between males with (\( n = 5 \) with black, 4 with white) and without (\( n = 4 \)) stickers masking the purple-black and blue-green patches on their eyespots (Dunnett’s tests, \( P > 0.36 \) for all comparisons). Thus, there is no evidence to suggest that the stickers were placed on males that would have been predicted to have lower mating success, on average.

The application of stickers to the males’ eyespots had no appreciable effect on their attendance on the lek (Figure 2.5c), or the amount of time they spent preening (Figure 2.5d). Although we frequently observed males attempting to remove stickers, the stickers did not appear to influence the amount of time they devoted to preening or lek attendance (Dunnett’s tests, \( P > 0.59 \) for all comparisons).
FIGURE 2.4 Partial regression plots showing effects of different eyespot colours on peacock copulation success. These graphs show the residual copulation rate from the best ‘all variables’ regression model (Table 2.1) plotted against residuals of latitudinal (\(\phi\)) and longitudinal (\(\theta\)) components of hue of the blue-green (BG) patch illuminated at 30° and 60°, controlling for the number of eyespots. Symbols indicate populations from which the data were collected.
FIGURE 2.5 Comparison of morphologies and behaviours of males with white \((n = 4)\), black \((n = 3)\), and no \((n = 4)\) stickers applied to all of their eyespots. Triangles indicate mean values; asterisks at the bottom of each pane indicate that the treatment is significantly different from the control (no stickers) by Dunnett’s test.
This colour manipulation resulted in a large decrease in male display rate, but the difference between control and experimental males in residual display rate (controlling for female visitation rate) was not significant (Dunnett’s tests, \( P > 0.07 \); Figure 2.5f). Female visitation rates (Figure 2.5e) were significantly lower for white- (\( P = 0.04 \)) but not black-stickered males (\( P = 0.19 \)), with the average female visitation rate for males without stickers nearly 4 times that of white-stickered males. The application of stickers to eyespots also resulted in a decrease in the rate of train rattling bouts (Figure 2.5g), though the differences between males with and without stickers were not quite significant (Dunnett’s tests, \( P > 0.10 \)). The rate of train-rattling bouts may be an indication of male attractiveness to females, as train-rattling displays always precede copulation attempts (Dakin and Montgomerie 2009).

Copulation attempt rates (Figure 2.5h) were substantially lower for stickered males, with the average attempt rate for males without stickers more than 8 times that of males with stickers. This difference was significant for white-stickered (Dunnett’s test, \( P = 0.03 \)) but not black-stickered males (\( P = 0.11 \)). Most important, males with eyespot colours hidden by stickers had a significant reduction in copulation rates relative to males with natural eyespot colours (Dunnett’s tests, \( P = 0.04 \) for black stickers, \( P = 0.02 \) for white; Figure 2.5i), with all four white-stickered males and four of the five black-stickered males achieving no copulations at all (and only one of four males without stickers failing to copulate). The average copulation rate of males without stickers was more than 3.5 times that of the lone black-stickered male who obtained a copulation.
The large effect sizes in comparisons between males with and without stickers indicate that females were less interested in visiting, being courted by, and copulating with males whose eyespot colours were not visible (see Table A2 in Appendix A).

2.5 DISCUSSION

A large proportion of the variation in peacock mating success in the feral populations we studied can be explained by the plumage colours of the males’ eyespots illuminated at angles typical of those during male courtship displays. Indeed, controlling for the potentially slight effect of eyespot number, which was primarily due to the experimental removal of >20 eyespots from some males, the colours alone accounted for half of the variation in peacock copulation success in our study populations. To the best of our knowledge this is one of the largest effects of an ornamental trait on reproductive success that has been documented in birds. Given that our estimate of copulation success is based on sampling a relatively small proportion of the time that each male spent courting, it is quite likely that some of the unexplained variation is due to sampling error. Thus, as supported by the results of our manipulative experiment, we conclude that peacock eyespot colours have a major influence on male mating success, and probably on male fitness as well, since paternity can be predicted from mating success in other birds with similar mating systems (e.g., Reynolds et al. 2007).

Our findings therefore confirm and extend the results from a study of feral peafowl in France (Loyau et al. 2007) where male copulation success was correlated with measures of brightness and iridescence taken from the blue-green eyespot patch. Loyau et al.
(2007) do not explain why they measured only the blue-green colour on the eyespot, but their choice was a good one, as we have shown that the other colours have little or no apparent influence on female choice (Table 2.1). In their study, iridescence of the blue-green patch—measured as the maximal change in chromatic contrast against a constant background with changing angle of reflectance (or measurement)—explained about 25% of the variation in male copulation success (data extracted from their Figure 4), as did brightness of that patch. Our measures of colour and iridescence explained approximately twice as much of the variation in male success, possibly because we used somewhat different measures of iridescence, or because we had a larger sample of males and observations. Another reason for the larger effect reported here might be that our measurements more accurately represented male colour variation, either because of the measurement geometry we used, or because our apparatus was designed to minimize inconsistencies in the position and distance of iridescent samples relative to the measurement probes. Interestingly, Loyau et al. (2007) did not find that the number of eyespots in the male’s train had any significant effect on male mating success once they controlled for eyespot colour (see also Dakin and Montgomerie 2011).

Our findings have several implications. First, we evaluated male colours at illumination angles (Figure 2.1) typical for the precopulatory train-rattling display (Dakin and Montgomerie 2009), and found a strong relation between mating success and the eyespot colours seen at this viewing configuration. Additional work is still needed to establish why males orient their trains relative to the sun in this way. It should be noted that the measurement geometries used in the present study do not capture everything that goes on
during peacock courtship displays (e.g., the ability of males to turn relative to the female and adjust the vertical tilt of train ornament). Measuring a single feather from each male cannot capture additional iridescent effects that may occur across the large hemispherical train. In addition, the motion of the train feathers during the train-rattling display may produce other visual effects not captured here. Nevertheless, the fact that about 50% of the variation in male mating success can be explained by these colour variables (Table 2.1) supports the notion that females choose males based on eyespots illuminated at those angles. Overall, our findings indicate that a better understanding of the selective pressures shaping iridescent colour signals in other animals can be achieved by considering viewing geometry and receiver perception when measuring these signals.

Second, our study of multiple populations builds on the findings of Loyau et al. (2007) to suggest that peahens in separate feral populations in North America and in France use similar criteria when evaluating potential mates. An increasing body of evidence demonstrates that female mate preferences are often quite variable, even within a single population (e.g., Chaine and Lyon 2008; see also Jennions and Petrie 1997), and it has been suggested that females in different feral peafowl populations may diverge in their preference for eyespot number (Loyau et al. 2008) or other ornamental traits. However, the cross-population consistency reported here suggests that peahens may have at least some universal preferences for the iridescent eyespot colours.

Third, we found very little evidence that the other two colour patches in the eyespot have any influence on male success (Table 2.1). Our analyses suggest that both the saturation
and iridescence of the bronze-gold patch might have a small effect, but the purple-black patch seems not to be a useful component of this ornament. Previously, a number of authors have suggested that the pattern elements of bird colouration are important social signals (e.g., Ferns and Hinsley 2004; Bortolotti et al. 2006). Although comparative studies of the evolution of ornamental plumage have considered multiple colour patches on different body regions (e.g., Endler et al. 2005; Stoddard and Prum 2008), our study is the first to consider how multiple colours on a single ornamental structure influence mating success. Our results lead to additional questions: Why does the eyespot contain three colour patches if two of them serve no apparent purpose? One possibility is that the purple-black and bronze-gold patches are incidental byproducts of the production of the blue-green patch during development. Another is that those two patches are the ghosts of sexual selection past, no longer having much influence on female choice. It is also possible that the bronze-gold and purple-black patches are reference colours used in the assessment of the blue-green patch (e.g., “attractive amplifiers” in Castellano and Cermelli 2010). Further experiments that mask or alter the colours of these two patches could help address this.

While this study provides strong support for female choice based on male eyespot colouration, many questions remain. Why do train-rattling peacocks orient relative to the sun, and how does this behaviour affect female perception of the males’ displays? Is the 45° illumination angle more informative for females than other angles? How does the train-rattling motion affect how females see and respond to those colours? Are subtle
changes in the angle at which a male presents his train important, and are eyespots illuminated from the right of the female preferred over those illuminated from the left?

Furthermore, what is the effect of altering particular colour patch attributes, rather than masking entire patches of colour, on female choice? The black and white stickers we used here had the effect of altering male appearance well beyond the natural range of variation. So far, no method has been developed to mimic or alter iridescent plumage colours in birds, analogous to the experimental alteration of pigment-based colours (e.g., Hill 1991). In a letter to J. J. Weir, Darwin pondered that “It wd be a fine trial to cut off the eyes of the tail-feathers of male-peacocks, but who wd sacrifice the beauty of their bird for which reason to please a mere naturalist!” (Darwin 1868). There is clearly much untapped potential for the peacock to tell us more about sexual selection.

2.6 REFERENCES


Darwin C. 1860. Correspondence Project Database, letter no. 2743. See http://www.darwinproject.ac.uk/entry-2743


CHAPTER 3. How lekking females decide to visit males: both male traits and a female’s prior experiences matter

3.1 ABSTRACT

Females often spend several days visiting and being courted by males, and while this behaviour has been characterized as a strategic process of sampling potential mates, we know relatively little about how it relates to mate choice. In this study, we describe the mate sampling behaviour of peahens (*Pavo cristatus*), and show that they bypass or skip about half of the displaying males they encounter. At the same time, they often pay repeat visits to particular males, even after copulating with them. We used observations of these repeated interactions to address the question of how females decide whether to visit, or skip, the males they encounter based on their traits and females’ prior experiences.

Females appear to remember the males they visit: first, they tend to spend more time viewing a male with each additional visit. Second, they tend to narrow the scope of their sampling efforts with time, suggesting that repeat visits are not chance encounters. Third, females appear to use different cues when deciding whether to visit males for the first time compared to subsequent encounters. For example, we show that male plumage colours only affect this decision when a female has visited the male in question at least once in the past, suggesting that females remember and return to certain males with more attractive colours. Finally, we also show that peahens tend diverge more from their previous travel path when they are travelling towards a male that they visit, relative to other encounters. Overall, these results suggest that females have the capacity to remember the males they visit, and that they use this information to guide their sampling and courtship decisions on leks.
3.2 INTRODUCTION

In a number of animal species including insects (Reid and Stamps 1997), crustaceans (Backwell and Passmore 1996), fish (Forsgren 1997), frogs (Arak 1988), birds (Trail and Adams 1989) and mammals (Byers et al. 2005), females visit several different males sequentially for courtship prior to mating. Both monogamous (Choudhury and Black 1993) and polygynous (Pruett-Jones and Pruett-Jones 1990) females do this, and it is generally thought that the function of this mate sampling process is that it allows females to evaluate and perhaps compare potential mates, for instance by assessing their traits, their courtship displays, or the quality of their territories (Janetos 1980; Andersson 1994), ultimately arriving at an adaptive mate choice. Thus, the sampling process may provide a window onto mate choice and the mechanisms involved.

The theoretical literature has identified two general classes of sampling strategies that females may use: in “threshold-based” strategies, females judge males against an internal standard, whereas in “best-of-n” or “sample-based” strategies, females visit and compare a number of different potential mates, and then return to their preferred choice for mating (Janetos 1980; Wittenberger 1983; Real 1990; Valone et al. 1996; Luttbeg 1996). In general, it is thought that threshold-based strategies may be favoured when searching is costly (Janetos 1980; Janetos and Cole 1981; Real 1990; Hutchinson and Halupka 2004), although more recent theoretical models that incorporate more realistic assumptions about the information acquired during sampling (e.g., Luttbeg 1996; Mazalov et al. 1996; Wiegmann et al. 2010), time vs. accuracy trade-offs, and time constraints (e.g., Luttbeg 2002; Castellano and Cermelli 2011) suggest that this may not always be the case.
Traditionally, empirical researchers have attempted to match observed patterns of mate sampling behaviour to these broad theoretical categories by examining the typical sequence of events when females visit and sample males, and have concluded that females use threshold-based (e.g., Gibson 1996; Backwell and Passmore 1996; Forsgren 1997; Reid and Stamps 1997) or sample-based (e.g., Trail and Adams 1989; Rintamäki et al. 1995; Fiske and Kålås 1995; Uy et al. 2001a) strategies based on the extent of their sampling and the frequency of repeat visits. The latter phenomenon, in which a female returns to a specific male that she has visited in the past, has generally been considered to be diagnostic of sample-based mate choice where the female remembers and compares visited males (Janetos 1980; Wittenberger 1983; Trail and Adams 1989; Reid and Stamps 1997). Repeat visits are common among many polygynous species (e.g., Pruett-Jones and Pruett-Jones 1990; Gibson 1996; Uy et al. 2001a; Byers et al. 2005; Draud et al. 2008).

Other authors have applied experimental approaches in the study of mate choice and mate sampling, examining female responses to different males (or their signals) presented under controlled conditions (e.g., Moore and Moore 1988; Zuk et al. 1990). For instance, in decorated crickets, where females typically encounter males sequentially in the wild, female responses in experimentally staged encounters with males are not affected by previous encounters with other males, regardless of how attractive those other males were (Ivy and Sakaluk 2007). Additionally, female field crickets – who prefer males with high chirp rates – apparently do not discriminate among different chirp rates when the options are all above or below a certain threshold rate (Beckers and Wagner 2011). Together
these studies suggest that crickets may base their mate choice decisions on a threshold that may be modified by experience.

Studies of natural mate choice in the wild emphasize that females often vary greatly in their sampling behaviour (e.g., Arak 1988; Hovi and Rättilä 1994; Dale and Slagsvold 1996; Murphy and Gerhardt 2002), making it difficult to draw direct connections with the theoretical literature on sampling strategies (Gibson and Langen 1996). For instance, females may adjust their mate sampling behaviour depending on events that occurred during the previous breeding season (Uy et al. 2000; 2001b), as well as current environmental conditions (Byers et al. 2006), and research also indicates that female responses to male signals can be shaped by females’ early-life experiences (e.g., Bailey and Zuk 2008). Another difficulty is that sample- and threshold-based mechanisms can produce similar behaviours, depending on a variety of other factors (Valone 1996; Wiegmann et al. 1996). The phenomenon of repeat visits, long considered to be diagnostic of a sample-based strategy (Janetos 1980; Wittenberger 1983; Trail and Adams 1989; Reid and Stamps 1997), could also occur for a variety of other reasons. Females could return to a male to assess the consistency of a dynamic trait (Getty 1996), for instance. In systems where males maintain consistent territories, females could return to certain males simply because they are located in areas that the females often visit, for instance if they are near foraging sites or other resources. One might even predict that females with the ability to remember and compare previously visited males would avoid repeat visits, to improve the efficiency of sampling (e.g., Mabry and Stamps 2008). Thus, it is not clear what we can conclude from the occurrence of repeat visits alone, although
they are likely a critical part of the process of gathering information about, or comparing, potential mates (Luttbeg 1996; Bateson and Healy 2005).

An alternative approach is to describe the mechanisms involved in natural mate sampling behaviour without attempting to test the fit of these behaviours to theoretical strategies. These include cognitive mechanisms such as signal detection, discrimination, and memory (Gibson and Langen 1996), which may be integrated when females decide whether to visit and mate with different males. To date there have been only a handful of studies of memory during mate sampling and mate choice (e.g., Wiegmann 1999; Akre and Ryan 2010), despite the fact that it is a key difference between threshold- and sample-based strategies, since only sample-based mate choice requires females to retain information about potential mates (Wittenberger 1983; Wiegmann 1999). Fewer still have considered the decision to visit males for courtship as being separate from mate choice itself (e.g., Murphy and Gerhadt 2002; Baugh and Ryan 2010). How do females decide to visit males? What role does memory play in this decision, and what does this imply about mate choice and the cognitive mechanisms involved?

3.2.1 Study system

We sought to address these questions using peafowl (*Pavo cristatus*), a lek-mating bird. In this species, males aggregate on display sites called leks that are visited by females solely for the purpose of courtship and copulation. Similar to other lek-mating species, peahens often visit several different males before copulation (Petrie et al. 1991). In a previous study, Petrie et al. (1991) proposed that peahens use a sample-based strategy of
comparing males using the number of eyespots displayed in their train ornaments. Petrie et al. (1991) based this on their observation that 10/11 females observed copulating did so at the end of a sequence of visits to different males, where the chosen male was the one with the most eyespots out of those visited. More recent work indicates that the number of eyespots displayed may not be the focus of mate choice (Takahashi et al. 2008), especially in populations where there is little variation among males in this trait (Dakin and Montgomerie 2011), and there is also recent evidence that peahens prefer certain males based on the brilliant iridescent colours of their eyespot feathers (Loyau et al. 2007; Chapter 2).

We used detailed observations of peafowl mate sampling behaviour to ask how females decide to visit males, and how they ultimately decide to accept or reject male copulation attempts, based on these traits thought to be important in mate choice. We also sought to test hypotheses about whether females remember and potentially compare the males that they visit. To do this, we applied two different observation methods in a single feral peafowl population. In 2009, we followed individual females continuously during their mate sampling forays to describe typical sampling behaviour. In 2010, we observed leks continuously to record all of the mate sampling and copulation events that occurred there involving a sample of males on those leks. Our aim was to analyze female sampling behaviour in relation to the traits of the males they encountered, as well as prior events they experienced during the mate sampling process. Lastly, we also examined the travel paths of mate sampling females on the leks to better understand the decisions involved.
3.3 METHODS

3.3.1 Field methods

We studied peafowl at the Los Angeles Arboretum in Arcadia, CA, USA, where there is a large free-range feral population that breeds in the Arboretum and surrounding residential areas. During the breeding season, there are several adjacent leks at the Arboretum, each with 1-6 adult males for a total of about 20 lekking males at this site (Figure 3.1). During the lekking season, adult peacocks maintain a display court of about 2-3 m$^2$ where they court females, and they tend to maintain the same display court year after year (R. Dakin, personal observations). Females visit the leks and observe male courtship displays, as they do in *P. cristatus* populations in the wild in India (Harikrishnan et al. 2010).

Peak lekking season for this population occurs in March, with most copulations happening mid- to late-March. We captured birds prior to the start of lekking activity in 2009 (Feb-March) and 2010 (Jan-March) and marked them with numbered leg bands (females: 13 in 2009, 35 in 2010 including 2 that were banded in 2009; males: 20 in 2009, 20 in 2010 including 13 that were banded in 2009). In 2009, we also observed 18 additional females that could not be captured, but that could be identified on the basis of unique crest and body plumage features.
FIGURE 3.1 Map of peacock display courts at the Los Angeles Arboretum. Asterix symbols denote the 16 display courts of males that were encountered by females in this study, with red symbols denoting the 11 courts on focal leks observed continuously in 2010. Line segments illustrate the frequency of trips taken by females observed in 2009, with line width proportional to the approximate frequency that each trip was observed. Note that not all possible trips between males occurred.
3.3.2 Observational methods

We observed females visiting males on leks at the using two different methods in two consecutive breeding seasons. In 2009, we used a focal female approach, tracking females individually during their mate sampling forays during peak lekking times (approximately 07:00-12:00 and 16:00-18:00 local time; Petrie et al. 1991; R. Dakin, personal observations) for a total of 121 h over the period of Mar 16-Apr 7. During this time, we observed a total of 23 copulation events among the 16 males that the females visited; of these copulations, 16 involved identifiable females (the rest involved unmarked females that could not be uniquely identified on the basis of plumage or other morphological features). In 2010, we used a focal male approach, observing 4 different leks continuously from 08:00-18:00 local time for a total of 506 h over a 13-day period (Mar 15-27), observing 19 copulation events among 11 males, 17 of which involved identifiable females.

3.3.3 2009 Focal female observations

To observe female mate sampling bouts, we walked between lek sites and selected the first female that was seen either standing or walking on one of the leks shown in Figure 3.1. We then followed that female from approximately 20 m away, recording which lek she was on, her general behavior (categorized as sitting, standing, moving, foraging, viewing a male, or preening), the identity of adult male closest to her on the lek, the approximate distance between the female and that male (estimated to the nearest m), and whether that male’s train was erect, and, if so, his train display behaviour (wing-shake, train-rattle, or none; see Dakin and Montgomerie 2009). We recorded these observations
and the time whenever there was a change in either the female’s behaviour or the identity of male closest to her. We also recorded all copulation attempts and successful copulations witnessed during our observation periods, regardless of whether the focal female was involved. We stopped observing a focal female when she had left the leks and had been sitting and stationary for > 5 min. On average, focal female watches lasted 56 min (95% CI 49-64, range 10-205 min, n = 123 watch periods), and in total, we observed 26 different females, including 8 that were marked and 18 were not marked but that could be identified. These data provide an indication of female behaviour and movement patterns during typical mate sampling forays; however, it should be noted that focal females may have also visited leks during times when we were not observing them.

3.3.4 2010 Focal lek observations

In 2010, 4 stationary observers monitored 4 leks continuously as described in 3.3.2. For each female that arrived on these leks, we recorded her general behaviour (sitting, standing, moving, foraging, viewing a male, or preening), the identity of the adult male closest to her on the lek, the approximate distance between the female and that male (m), whether that male’s train was erect, and his display behaviour, as we did during focal female observations. Again, we noted these observations and the time whenever there was a change in either the female’s behaviour or the identity of the male closest to her. We also recorded all copulation attempts and copulations that occurred on the leks observed. Because there were often multiple males and females present on the lek simultaneously, we were not able to consistently identify unmarked females in these focal lek observations; thus, our sample here was limited to 20 marked females.
In contrast with the focal female observations above, these methods provide a higher degree of certainty that we witnessed most or all female visits to the set of 11 males with display courts on the 4 focal leks we observed. However, these data do not represent complete female sampling forays. Additionally, since the females observed here almost certainly visited other males elsewhere at other times, these data do not encompass the full sequence of mate sampling events for each female.

### 3.3.5 Summarizing female encounter sequences

We summarized the sequence of observed events over the course of the season for each identifiable female in each year. We defined an “encounter” with a male as any period where a female was present on a lek and the male closest to her was an adult male attending his lek display court territory. If the male’s train was erect at some point during the encounter, we further characterized it as either a “visit” or a “skip” event. We defined a visit as an encounter where a female viewed a male from ≤ 2 m away while he performed his train-rattling display, whereas a skip was any encounter where the male’s train was erect, but the female did not visit him. Figure 3.2 summarizes these definitions. When a female viewed a male’s train-rattle in multiple discrete bouts over a single encounter (i.e., she did not approach closer to and thus encounter any other adult male in between viewing bouts), we counted it as a single visit event. For each visit, we calculated the total time spent near the visited male, as well as the total time spent viewing the visited male’s train-rattling display as the sum of the viewing times for any discrete viewing bouts.
FIGURE 3.2 Schematic and definitions of behaviours during peafowl mate sampling.
In our 2009 focal female observations, we witnessed a total of 431 encounters, with an average of 17 encounters per female (95% CI 9-24, range 1-89, n = 26 females), and an average of 6 visits (95% CI 3-9, range 0-31) and 7 skips (95% CI 3-12, range 0-48) per female. In our 2010 focal male observations, we witnessed a total of 624 encounters, with an average of 31 encounters per female (95% CI 14-48, range 1-121, n = 20 females), and an average of 11 visits (95% CI 5-16, range 0-36) and 17 skips (95% CI 7-27, range 0-73) per female.

Overall, about 40% of encounters were visits on average, per female (2009: 41%, 95% CI 30-52, range 0-1; 2010: 37%, 95% CI 25-49, range 0-1), and between 40-50% were skips (2009: 40%, 95% CI 30-50, range 0-1; 2010: 51%, 95% CI 39-62, range 0-1); the rest were males encountered with trains that were not erect. The proportion of visits and skips per female did not significantly differ between years (t-tests; proportion visits: $F_{44,1} = 0.27$, $p = 0.60$; proportion skips: $F_{44,1} = 2.18$, $p = 0.15$).

We characterized all encounters according to females’ prior experiences with the encountered male. We defined a female as being “informed” about a particular male if she had previously visited him (i.e., she had viewed his train-rattling display during a previous encounter), and “uninformed” if she had not been seen doing so. Visits were also characterized as being “first” or “repeat” visits, depending on whether we had previously witnessed that female visiting that particular male. We also determined the ordinal numeric sequence of a female’s observed visits to each unique male that she
visited (such that her first visit to a male that we observed would be numbered 1, and her second visit to that male 2, and so on).

Because we did not observe all females at all times continuously, these data do not necessarily encompass all visits for a given female in the season that she was observed. Visits may have occurred at other times when focal females were not being observed in 2009, and the females we observed in 2010 most likely visited other leks as well. Thus, our category of uninformed females likely includes some that were in fact informed (i.e., they had visited the male in question but we did not observe it), and events that we classified as first visits may have been repeat visits. However, these categories should provide a reasonable estimate of the extent of a female’s prior experiences with particular males, especially given that the reverse error of classifying an uninformed female as being informed should not have occurred.

3.3.6 Male ornamental traits

To investigate how females make sampling and mate choice decisions, we considered measures of male train ornamentation that have previously been shown to correlate with male mating success, including train length (Yasmin and Yahya 1996), the number of eyespots displayed in the train (Petrie et al. 1991; Dakin and Montgomerie 2011), and the colours of the eyespots as they would be perceived by females (Loyau et al. 2007; Chapter 2). Train length was measured as the length (cm) of the longest fish-tail feather in the train (Petrie et al. 1991). To assess eyespot number, we digitally photographed
displaying males during the breeding season and counted the number of eyespots displayed (Dakin and Montgomerie 2011).

To quantify male colours, we selected the single most symmetrical eyespot from a set of 5 of the longest major eyespot feathers that were sampled from every captured male (Dakin and Montgomerie 2011). These were stored in a paper envelope prior to taking colour measurements in a darkroom. We used a USB4000-UV-VIS spectrometer and a DH-2000 Deuterium Tungsten Halogen light source (Ocean Optics, Dunedin, FL, USA) to measure the blue-green and bronze colour patches on the right side of each eyespot (facing the feather) at three light angles, setting the illumination probe at 30°, 45° and 60° to the feather surface, to approximate light conditions during typical peacock courtship displays (Dakin and Montgomerie 2009).

Following Stoddard and Prum (2008), we calculated perceived colour variables using a tetrahedral colour space model of avian colour perception based on the retinal cone sensitivities for peafowl reported in Hart (2002). We defined “eyespot iridescence” as the perceived chromatic contrast of the blue-green patch (relative to itself) over a change in illumination angle from 60° to 30°, and we defined “eyespot colour contrast” as the perceived chromatic contrast between the blue-green and bronze patches illuminated at 60°. Further details on colour measurements and methods of modeling perceived colour contrast are described in Chapter 2.
In total, we considered four variables describing male ornamental traits: train length (cm), the number of eyespots displayed, eyespot iridescence, and eyespot colour contrast.

3.3.7 Female movements between males

We used the geographical coordinates of male display courts to determine the approximate movement patterns of females that we followed during their mate sampling forays in 2009. Although we did not record detailed travel paths of females as they moved about on the leks, we noted that the females generally approached encountered males to within a few metres on their display courts, and we estimated the mean distance of closest approach as 4.7 m ± 0.79 SE (estimated as the intercept in a mixed model with female and male identity as random variables, n = 1055 observations of 44 females encountering 16 males). We therefore used data on female encounter sequences and the geographical coordinates of male display courts to estimate female travel paths during their mate sampling forays.

To do this, we first identified the precise location of each male’s court relative to local landmarks in satellite images and extracted coordinates for its latitude and longitude (Google Maps, 2012). These data corresponded to GPS coordinates collected in the field (Magellan eXplorist 210, position accuracy 7 m), but were more accurate than the GPS device, which identified neighbouring courts within 20 m of one another as having the same coordinates. We used latitude and longitude coordinates to estimate the approximate distance (to the nearest m) and compass bearing for every possible straight-line trip between two display courts, using the spherical law of cosines with a radius of
6371 km for the earth to convert latitude and longitude to Cartesian coordinates. The average between-court distance for the 16 courts studied was 66.3 m (95% CI 60.3-72.3, range 9.4-162.4, n = 120 pairwise distances; Figure 3.1).

We then determined the travel paths of females during their mate sampling forays, assuming straight-line trips between male encounters. Figure 3.3 illustrates some typical sampling forays by females observed in 2009. We excluded any trips where the female did not approach one of the encountered males to within ≤ 10 m (n = 28/299 trips excluded). For forays that included ≥ 2 trips, we also calculated the approximate change of course between sequential trips as the absolute value of the difference in the female’s bearing. This variable was bounded between 0º and 180º, where 0º represents no course change (i.e., continuing on straight ahead), and 180º represents the maximal course change possible (i.e., returning to the last encounter location). We also converted change of course to a binary response based on whether it was ≥ 90º.

Male display courts tend to be more tightly clustered in certain areas of the leks (Figure 3.1), and females might encounter some males more often simply because they are centrally located. Thus, for each male court we also calculated its “average neighbour distance” as a measure of its centrality, by taking the average distance for all possible straight-line trips between that display court and each of the other 15 courts studied (Figure 3.1). This measure is negatively related to centrality: peripheral courts have a greater average neighbour distance than ones that are centrally located (e.g., the display court at the top of Figure 3.1 has the greatest average neighbor distance).
FIGURE 3.3 Sampling forays by 8 different peahens observed in 2009 (see following page for additional images). Asterix symbols denote male display courts, and female encounters with males are numbered in the order they occurred. Colours indicate the type of the encounter (black = the male was encountered with his train down; red = skip; green = visit; purple = visit with copulation). Arrows indicate the direction of female trips between male encounters. The date, start time, and end time for the observation periods are given in the upper left of each map.
FIGURE 3.3 cont. Sampling forays by 8 different peahens observed in 2009. Asterix symbols denote male display courts, and female encounters with males are numbered in the order they occurred. Colours indicate the type of the encounter (black = the male was encountered with his train down; red = skip; green = visit; purple = visit with copulation). Arrows indicate the direction of female trips between male encounters. The date, start time, and end time for the observation periods are given in the upper left of each map.
3.3.8 Data analysis

Analyses were performed using JMP 9.0.0 and R 2.14.1 (R Development Core Team 2011). To analyze female mate sampling behaviour, we used generalized linear mixed models (GLMMs) where we included both female and male identity as separate random variables to account for non-independence of repeated observations of the same individual. We fit these models using the R package lme4. We analyzed female decisions to visit or skip encountered males, and to accept or reject their copulation attempts, as binary responses in GLMMs with binomial error distributions and the logit link. We used a Gaussian error distribution and the identity link when analyzing females’ change of course, because while these data were bounded, the residuals from the Gaussian model appeared to be normally distributed and consistent across different predictor variables. We also report results from a binomial GLMM modeling females’ change of course as a binary variable. Continuous data describing the amount of time females spent near visited males and viewing their train-rattling displays as well as the distance that females travelled between males appeared to fit a gamma distribution. To model these data, we fit GLMMs with a gamma distribution of errors and the log link using penalized quasi-likelihood in the MASS package in R. We included a random effect for female identity only in these models, since the glmmPQL function we used to fit them cannot account for multiple random effects. We also checked these gamma models using male identity as a random effect instead (and omitting female identity), and found similar results.

In all models, we controlled for the effects of year and date. Following Bolker et al. (2009) we report Wald tests of fixed effects in binomial and gamma mixed models and
likelihood ratio tests of fixed effects in Gaussian mixed models. We used the R package
languageR 1.0 to estimate effect sizes in terms of the change in the response variable over
the range of a given predictor, assuming median values for the other predictors in a mixed
model.

3.4 RESULTS

3.4.1 Description of female mate sampling

Females were observed visiting leks over a span of about 7-8 days on average (Table
3.1). Both of the observation methods we used yielded similar results in terms of the total
number of unique males encountered (~6) and visited (~3-4) by each female per year on
average (Table 3.1), despite the fact that our focal lek observations in 2010 were limited
to a smaller number of males (11 vs. 16 males encountered by females in 2009). We
observed females revisiting about 1-2 different males per year, on average, and they
tended to do so repeatedly, since the number of revisit events per female was generally
greater than the number of males revisited (Table 3.1). This can also be seen in Figure
3.3, which illustrates sampling sequences for 8 females that were observed in 2009 and
2010.
TABLE 3.1 Mate sampling on peafowl leks. In 2009, individual females were followed for the duration of their sampling forays (n = 26 females), whereas in 2010 females were observed during continuous observations of focal lek sites (n = 20 females).

<table>
<thead>
<tr>
<th></th>
<th>2009 focal female observations</th>
<th>2010 focal lek observations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>total no. male encounters</td>
<td>16.6</td>
<td>8.9-24.3</td>
</tr>
<tr>
<td>no. visits</td>
<td>6.0</td>
<td>3.3-8.8</td>
</tr>
<tr>
<td>no. revisits</td>
<td>2.7</td>
<td>0.7-4.6</td>
</tr>
<tr>
<td>no. skips</td>
<td>7.5</td>
<td>3.3-11.6</td>
</tr>
<tr>
<td>unique males encountered</td>
<td>6.5</td>
<td>4.9-8.1</td>
</tr>
<tr>
<td>unique males visited</td>
<td>3.3</td>
<td>2.3-4.3</td>
</tr>
<tr>
<td>unique males revisited</td>
<td>1.3</td>
<td>0.6-1.9</td>
</tr>
<tr>
<td>proportion visits in the AM</td>
<td>0.50</td>
<td>0.33-0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no. sampling forays †</td>
<td>4.7</td>
<td>2.7-6.8</td>
</tr>
<tr>
<td>total length of foray (min)</td>
<td>52.9</td>
<td>40.0-65.7</td>
</tr>
<tr>
<td>visits per foray</td>
<td>1.4</td>
<td>1.0-1.8</td>
</tr>
<tr>
<td>days observed sampling on leks ‡</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>visits per day</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>span of days observed on leks</td>
<td>7.6</td>
<td>4.6-10.6</td>
</tr>
<tr>
<td>typical encounter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>time spent near male (min)</td>
<td>10.1</td>
<td>8.1-12.2</td>
</tr>
<tr>
<td>typical visit §</td>
<td></td>
<td></td>
</tr>
<tr>
<td>time spent near male (min)</td>
<td>14.1</td>
<td>10.1-18.1</td>
</tr>
<tr>
<td>bouts observing male</td>
<td>1.00</td>
<td>1.00-1.01</td>
</tr>
<tr>
<td>time observing train-rattle (min)</td>
<td>3.4</td>
<td>2.5-4.4</td>
</tr>
</tbody>
</table>

† not given for 2010 since observations were of focal leks, and entire female sampling forays were not recorded
‡ not given for 2009 since females may have sampled on days when they were not the subject of focal observations
§ n = 23 and 17 females for 2009 and 2010, respectively, as some females were not observed visiting any males
In an earlier study of feral peafowl, Petrie et al. (1991) also found that peahens pay repeat visits to certain males, and reported that females make about 5.4 visits per sampling bout (95% CI 2.5-8.2, range 2-14, n = 11 females on a lek of 10 males) to about 3 different males, on average (95% CI = 2.2-3.8, range 2-5). Petrie et al. (1991). These findings are generally consistent with our estimate of 3.3-3.7 for the average number of unique males visited per female (Table 3.1), although it should be noted that these are all likely to be underestimates, since not all visits were necessarily observed in either study, and since Petrie et al. (1991) observed each female on a single day and not over the extended period of mate sampling that we document here (Table 3.1).

In our study, focal lek observations yielded higher estimates than focal female observations for the total number of encounters, visits, revisits and skips per female (Table 3.1). This was most likely due to a difference in sampling effort per female, since in 2009 no more than two females were observed at a time. Thus, females in 2009 may have visited leks at times when they were not under observation. Nevertheless, the consistency in the average number of unique males visited per female indicates that females may limit their sampling to a relatively small subset of the males they encounter. Additionally, with both methods, we found that females skipped around 50-60% of the displaying males they encountered, on average (2009: mean = 50%, 95% CI = 38-63, range 0-100, n = 26; 2010: mean = 59%, 95% CI 46-72%, range 0-100, n = 20).

Females generally spend more time near a male during a visit, relative to other encounters (Table 3.1). When females visit males, they will view his train-rattling display
for about 2-3 minutes on average, although this can go on for much longer (up to 16 minutes; see Figure 3.4). Our focal female observations yielded considerably higher estimates for time spent near males and time spent viewing males on average, relative to the focal lek observations. This may have been due to our method of sampling, since in 2009 focal females were selected because they were the most active on leks at the time, whereas in 2010 our focal lek observations include any females that were present on the focal leks, including those females that may have been more motivated to engage in other activities (e.g., foraging). In addition, 2009 focal female observations were collected only during peak lekking times in the morning and late afternoon, whereas 2010 focal lek observations were conducted continuously and include the mid-day period when lekking activity is reduced (Petrie et al. 1991; R. Dakin, personal observations), and when both males and females may be less motivated to engage in courtship.
FIGURE 3.4 Amount of time females spent: (a) near visited males, and (b) viewing male train-rattling displays, in relation to their sequence of visits to those males. Visit sequence is a measure of the females’ level of prior experience and familiarity with visited males.
3.4.2 Description of copulation behaviour

Most of the identifiable females that we observed (35/46) were never seen copulating. Of the females that were observed mating, most (9/11) were seen mating multiple times (mean no. cops = 3, 95% CI 2.2-3.8, range 1-5), which is considerably greater than Petrie et al.’s (1992) estimate that about 50% of peahens in their study mated multiply. In our study, all of the females that mated multiply returned to copulate again with a previous mate, consistent with what Petrie et al. (1992) observed. Additionally, we observed that the majority of copulations (22/33) occurred during a revisit event (i.e., when a female was visiting a male that she had previously viewed). This estimate is also potentially a conservative one, since females may have visited the chosen males at other times that we did not observe.

Nearly all of the identifiable females seen copulating (10/11) continued to visit males after their first copulation (mean no. post-copulatory visits = 13.9, 95% CI = 6.4-21.4, range 0-32), including visiting potentially new males that we had not seen them visiting before the copulation. See Appendix B for figures illustrating the encounter sequences for 8 of the females that we observed copulating in 2009 and 2010. The mean number of new males visited after a female’s first copulation was 2.9 (95% CI 1.8-4.0, range 0-6), although it is possible that some or all of these males were in actual fact ones that the females had previously visited. Overall, it is clear from these results that females continue to sample males post-copulation, and that both courtship and copulation occur frequently throughout the sampling period (see also Appendix B).
3.4.3 Diel and seasonal patterns

Copulations tended to peak in late March (mean for both years pooled = March 24, range = March 15-April 6, n = 33; Figure 3.5), although dates for copulation events were not normally distributed. On leks observed continuously in 2010, visit and copulation dates were bimodally distributed (Figure 3.5), and we confirmed this with Hartigan’s dip test for visit dates (D = 0.09, p < 0.0001, n = 214), although the smaller sample of copulation dates did not significantly differ from unimodality (D = 0.11, p = 0.06, n = 17). We could not test for bimodality at the level of individual females because we did not have complete female sampling sequences. However, we visually inspected distributions of visit dates for 13 females that were observed in 2010 on more than one occasion over a span of ≥ 3 days, and we noted that visit dates for 8/13 of these females appeared to be bimodally distributed, with a span of about 5 days on average where the female was not seen visiting males (95% CI = 3.8-6.4, range 2-7, n=8). This is similar to what Uy et al. (2001a) observed in their study of mate sampling in bowerbirds, where females sampled males in two distinct bouts with a period of about 7 days in between.

Controlling for study year, females were more likely to visit a male encountered with his train erect (rather than skip him) earlier in the season (date: b = −0.05, SE = 0.02, Z = 2.62, p = 0.009), but no more likely to do so in the morning than the afternoon (AM vs. PM: b = 0.06, SE = 0.16, Z = 0.40, p = 0.69; n = 902 encounters between 44 females and 16 males). The coefficient for date in this model corresponds to a 25% decline in the probability that a female will visit an encountered male on April 5 vs. March 15, all else being equal.
FIGURE 3.5 Dates for visit and copulation events observed in (a) 2009 and (b) 2010 at the Los Angeles Arboretum.
Copulations occurred in both the morning and afternoon, with 18/33 or about 54% occurring before noon. We found no effect of date ($b = -0.11$, SE = 0.08, $Z = 1.39$, $p = 0.17$) or time of day, comparing morning and afternoon times (AM vs. PM: $b = -0.14$, SE = 0.68, $Z = 0.21$, $p = 0.84$), on the probability that a female would accept a male’s copulation attempt ($n = 86$ attempts on 25 females by 11 males).

### 3.4.4 The decision to visit a male

We examined the effect of a female’s prior experiences and the effect of the traits of the males she encountered on her decision to visit those males. We first found that females were generally more likely to visit males they had visited in the past, rather than skip them ($b = 0.39$, SE = 0.17, $Z = 2.29$, $p = 0.02$, $n = 902$ encounters between 44 females and 16 males), controlling for year and date. The coefficient in this model corresponds to a 10% increase in the probability of visiting a familiar male, all else being equal. When incorporating male traits into this model, we found evidence of interactions between a female’s prior experience of a male and three of the four male traits we measured (including iridescence, colour contrast and number of eyespots; $p < 0.10$ for interaction effects), so we separately modeled the outcome for informed and uninformed females.

For uninformed females that had not previously viewed the male they were encountering, only the length of the male’s train affected the probability that the female would visit him (Table 3.2). The coefficient for train length in this model corresponds to a 21% increase in the probability that females would visit males with the longest trains in our study.
population, compared to those with the shortest trains, upon encountering a given male for the first time.

In contrast, train length did not affect the probability that an informed female would revisit a particular male that she had viewed before (Table 3.3). Instead, the probability of revisiting a male was affected by the number of eyespots displayed, eyespot colour contrast, and eyespot iridescence (although the effect of iridescence was not significant). Coefficients in this model correspond to a 54% increase in the probability that a female would revisit a male with the fewest eyespots, a 58% increase in the probability that she would revisit a male with the greatest eyespot colour contrast, and a 26% increase in the probability that she would revisit a male with the greatest iridescence, relative to a male with the lowest value for iridescence in our study population, all else being equal.
TABLE 3.2 Factors affecting the decision to visit a male, when the female has not previously viewed him (i.e., she is not informed about the male she is encountering). Statistics are reported for Wald tests of fixed effects in the final model.

<table>
<thead>
<tr>
<th>n</th>
<th>obs.</th>
<th>♂</th>
<th>♀</th>
<th>estimate</th>
<th>SE</th>
<th>Wald Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>uninformed</td>
<td>424</td>
<td>44</td>
<td>16</td>
<td>intercept</td>
<td>−1.92</td>
<td>4.41</td>
<td></td>
</tr>
<tr>
<td>fixed effect</td>
<td></td>
<td></td>
<td></td>
<td>year</td>
<td>0.28</td>
<td>0.33</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>date</td>
<td>−0.03</td>
<td>0.03</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>train length</td>
<td>0.03</td>
<td>0.01</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no. eyespots displayed</td>
<td>−0.01</td>
<td>0.02</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>eyespot iridescence</td>
<td>5.49</td>
<td>7.13</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>eyespot colour contrast</td>
<td>3.67</td>
<td>3.08</td>
<td>1.19</td>
</tr>
<tr>
<td>random effect</td>
<td></td>
<td></td>
<td></td>
<td>female identity</td>
<td>variance</td>
<td>0.24</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>male identity</td>
<td>SD</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
TABLE 3.3 Factors affecting the decision to revisit a male that had been visited previously. Females were considered informed about the male they were encountering if they had previously been observed viewing his train-rattling display. Statistics are reported for Wald tests of fixed effects in the final model.

<table>
<thead>
<tr>
<th>n</th>
<th>estimate</th>
<th>SE</th>
<th>Wald Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>informed♀</td>
<td>478</td>
<td>30</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>fixed effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>year</td>
<td>0.59</td>
<td>0.35</td>
<td>1.71</td>
<td>0.09</td>
</tr>
<tr>
<td>date</td>
<td>–0.08</td>
<td>0.02</td>
<td>3.04</td>
<td>0.002</td>
</tr>
<tr>
<td>train length</td>
<td>–3*10⁻⁵</td>
<td>0.02</td>
<td>0.002</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>no. eyespots displayed</td>
<td>–0.14</td>
<td>0.03</td>
<td>4.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>eyespot iridescence</td>
<td>12.8</td>
<td>6.8</td>
<td>1.87</td>
<td>0.06</td>
</tr>
<tr>
<td>eyespot colour contrast</td>
<td>17.0</td>
<td>3.6</td>
<td>4.75</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

| variance SD | female identity | <0.0001 | <0.0001 |
| male identity |            | 0.02    | 0.15    |
3.4.5 The decision to copulate

We tested the effect of male traits and females’ prior experiences on the decision to accept or reject male copulation attempts. With this smaller dataset (n = 86 attempts by 11 males on 25 females), we did not control for year and date, because models failed to converge with the large number of predictor variables. However, as noted above (3.4.3), we found no evidence that study year or date had an effect on females’ decisions to accept or reject male copulation attempts (all p > 0.16).

As shown in Table 3.4 and Figure 3.6, females were more likely to accept a copulation attempt from males with greater eyespot iridescence and greater eyespot colour contrast. This is consistent with our previous study demonstrating that male mating success is related to colour traits (Chapter 2). The coefficients in the model shown in Table 3.4 correspond to a 78% greater probability that a female will accept a copulation attempt by a male with the greatest eyespot colour contrast, and an 85% greater probability that she will accept a copulation attempt by a male with the greatest eyespot iridescence, relative to males with the lowest values we measured for these traits, all else being equal.
**TABLE 3.4** Factors affecting the decision to accept a male’s copulation attempt during a visit. Statistics are reported for Wald tests of fixed effects in the final model.

<table>
<thead>
<tr>
<th>n</th>
<th>obs.</th>
<th>♀</th>
<th>♂</th>
<th>estimate</th>
<th>SE</th>
<th>Wald Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>copulation attempt</td>
<td>86</td>
<td>25</td>
<td>11</td>
<td>−22</td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fixed effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female informed?</td>
<td>0.17</td>
<td>0.62</td>
<td>0.28</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>train length</td>
<td>−0.03</td>
<td>0.03</td>
<td>0.86</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no. eyespots displayed</td>
<td>−0.03</td>
<td>0.06</td>
<td>0.58</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eyespot iridescence</td>
<td>69.5</td>
<td>23.0</td>
<td>3.02</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eyespot colour contrast</td>
<td>35.3</td>
<td>12.7</td>
<td>2.77</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>random effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female identity</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male identity</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 3.6 Probability of accepting a copulation attempt in relation to (a) male eyespot colour contrast and (b) eyespot iridescence. Red lines illustrate the regression effects from the mixed model in Table 3.4. All raw data are shown; the size of the grey circles corresponds to the frequency of each data point.
3.4.6 Scope of mate sampling

To test whether females adjusted the scope of their mate sampling as time progressed, we examined the effect of previous events on the probability that they would visit new and previously viewed males. For a female encountering a new male (i.e., she was uninformed about him), the more times she had revisited other males, the less likely it was that she would visit him \((b = -0.08, \text{SE} = 0.03, Z = 2.22, p = 0.03, n = 424\) encounters between 44 females and 16 males), controlling for year and date. This coefficient corresponds to a decrease of about 10% in the probability of visiting a new male after 5 visits, all else being equal. In contrast, a female’s total number of previous revisit events does not affect the probability that she will revisit a male she has visited before \((b = -0.03, \text{SE} = 0.02, Z = 1.49, p = 0.14, n = 478\) encounters between 30 females and 13 males), controlling for year and date.

3.4.7 Time spent during visits

If females remember males during mate sampling, one might predict that their responses will change with level of familiarity. Thus, we investigated the effect of a female’s prior experiences with males on the length of time spent near and time spent viewing them during visits.

A female’s status as to whether she was informed about a particular male (i.e., whether or not we had seen her visiting him at least once) did not affect the amount of time she spent near him, nor did it affect the amount of time she spent viewing his train-rattling display (Table 3.5). In general, though, the more times a female had visited a male in the past, the
longer she tended to spend near him, and the longer she tended to spend viewing his train-rattling display, controlling for year and date (Figure 3.4). The coefficients from models shown in Table 3.5 indicate that these effects are subtle, with an approximate 6% increase in time spent near a male with each additional visit, and an approximate 7% increase in time spent viewing his train-rattling display per additional visit.
**TABLE 3.5** The effect of prior experience on time spent near males during courtship visits. A female was considered to be informed about a male if she had previously visited that particular male at least once. Visit sequence refers to the ordinal numeric sequence of a female’s visits to a particular male (increasing by 1 with each additional visit). Results are given for GLMMs with a gamma distribution of errors and the log link accounting for repeated observations of the same female. Statistics are reported for Wald tests of fixed effects.

<table>
<thead>
<tr>
<th>n</th>
<th></th>
<th></th>
<th>estimate</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>obs.</td>
<td>female (♀)</td>
<td>male (♂)</td>
<td>intercept</td>
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<td>1.03</td>
<td></td>
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<tr>
<td>time spent near male (min)</td>
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<td>38</td>
<td>15</td>
<td>fixed effect</td>
<td>year</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>date</td>
<td>0.03</td>
<td>0.16</td>
<td>2.29</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>informed ♂</td>
<td>–0.06</td>
<td>0.12</td>
<td>0.50</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>visit seq.</td>
<td>0.06</td>
<td>0.02</td>
<td>3.66</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>random effect</td>
<td>variance</td>
<td>female identity</td>
<td>0.17</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>time spent viewing T-R display (min)</td>
<td>369</td>
<td>38</td>
<td>15</td>
<td>fixed effect</td>
<td>year</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>date</td>
<td>–0.03</td>
<td>0.01</td>
<td>2.68</td>
<td>0.008</td>
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</tr>
<tr>
<td></td>
<td>informed ♂</td>
<td>0.02</td>
<td>0.11</td>
<td>0.16</td>
<td>0.88</td>
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</tr>
<tr>
<td></td>
<td>visit seq.</td>
<td>0.07</td>
<td>0.01</td>
<td>4.75</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
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<td>variance</td>
<td>female identity</td>
<td>0.01</td>
<td>0.0002</td>
<td></td>
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</tr>
</tbody>
</table>
3.4.8 Female movements between males

Figure 3.2 illustrates typical sequences of events during female mate sampling forays observed in 2009. In general, females tended to travel greater distances between male display courts later in the season, controlling for the effect of a court’s centrality (or average neighbour distance) on trip distance (Table 3.6). The coefficient for date in this model indicates that, all else being equal, females’ trip distances increased by a factor of about 1.6 over the 22 day period from March 16-April 5.

We also found that females tended to make substantially greater deviations from their previous course when travelling towards a visit, relative to other encounter types, controlling for the effect of court centrality (Table 3.6; Figure 3.7). We also modeled change of course as a binary variable, and found that females on their way to visit a male were 15% more likely to diverge more than 90° from their previous course heading (b = 0.63, SE = 0.36, Z = 1.74, p = 0.08) when controlling for date and male display court centrality (b = 0.06, SE = 0.02, Z = 3.69, p = 0.0002; n = 179 encounters of 21 females with 15 males).
TABLE 3.6 Female movement patterns in relation to mate sampling behaviour on peacock leks. Distance travelled was modeled using a gamma GLMM accounting for repeated observations of the same female, whereas change of course was modeled using a Gaussian GLMM accounting for repeated observations of females and males. Average neighbor distance (AN distance) is a measure of display court centrality, with centrally located courts having a lower average neighbor distance. Statistics are reported for Wald tests of fixed effects for the gamma model and likelihood ratio tests of fixed effects for the Gaussian model.

<table>
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<th>Variable</th>
<th>n</th>
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<th>obs.</th>
<th>estimate</th>
<th>SE</th>
<th>t</th>
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<td></td>
<td></td>
<td>visit ?</td>
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<td>0.08</td>
<td>0.65</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>variance</td>
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<tr>
<td><strong>Change of course</strong></td>
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<td>179</td>
<td>intercept</td>
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<td>76.02</td>
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<td>visit ?</td>
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FIGURE 3.7 Histogram showing females’ change of course in relation to the type of encounter (visit vs. non-visit). Non-visits include skips and encounters where the male’s train was not erect.
3.5 DISCUSSION

Peahens spend about a week visiting males on leks, paying multiple repeat visits to several males in a manner that is similar to the sampling behaviour observed in other lekking birds (e.g., Guianan cock-of-the-rock, Trail and Adams 1989; Lawes’ parotia, Pruett-Jones and Pruett-Jones 1990; great snipe, Fiske and Kålås 1995; black grouse, Rintamäki et al. 1995; sage grouse, Gibson 1996). They also avoid or skip about half of the displaying males they encounter on leks. We showed here that both a male’s traits and a female’s prior experience of those traits influence her decision of whether to visit him. Furthermore, by analyzing how individual females respond to male copulation attempts, and showing that a male’s iridescent plumage colours influence this decision, our results here support previous findings on mate choice in peafowl (Chapter 2; see also Loyau et al. 2007). At the same time, we found that a peacock’s eyespot plumage colours do not seem to influence the decision of whether to visit a male initially. This may be because females need to view males at close range, or for an extended period of time, in order to evaluate their eyespot colours.

These results also indicate that peahens remember the males they visit, consistent with a sample-based mechanism of mate choice. Specifically, we found evidence that when females encounter a male for the first time, the length of his train may influence their decision of whether to visit him, but not other aspects of train ornamentation. In contrast, in subsequent encounters, and when faced with a copulation attempt, females may be influenced by other traits, including the male’s eyespot colours. Thus, females may use different criteria when deciding to visit a male for the first time, compared to one they
have viewed in the past, suggesting that memory can affect their visit decisions. Our findings also provide two other lines of evidence that peahens remember males visited during mate sampling. First, we showed that females will generally spend more time near a male, and more time viewing his display, the more they have visited him in the past. This suggests that prior experience and level of familiarity may influence the courtship interaction. Second, our results indicate that repeat visits are not random encounters: peahens are more likely to visit males they have viewed in the past, and they tend to narrow the scope of their sampling efforts as they start to pay repeat visits to certain males, avoiding visiting new ones.

Our findings with respect to male ornamental traits are limited by the fact that this was an observational study. We chose to analyze a set of traits that had been shown to correlate with male mating success in previous studies, but peacocks have additional traits, calls, and courtship behaviours that we did not consider here, and that may also influence female visit and copulation decisions. The question of how females use these varied signals can only be resolved by further study, and ideally, experiments with controlled stimuli. Nevertheless, even if females use other correlated cues and not the traits measured here, our results still demonstrate that females use different criteria depending on their level of experience or familiarity with particular males. The use of multiple cues in a sequential or hierarchical fashion during mate choice has been proposed in other species (e.g., Borgia 1995; Backwell and Passmore 1996; Dale and Slagsvold 1996; Robson et al. 2005; Gomez et al. 2011), and other studies have also demonstrated that female responses to males can depend on recent experience with other males (e.g.,
Bakker and Milinski 1991). This is the first study to implicate memory for particular males as having an effect on the way females respond to them, and on the way females use their courtship signals.

Mate sampling on peafowl leks shows some interesting parallels to mate sampling in satin bowerbirds, another polygynous species. Uy et al. (2001a) showed that bowerbird females undertake two distinct bouts of mate sampling, with a period of several days in between where females may not visit males. It has been suggested that this may allow females gather additional information about particular males (Luttbeg 1996; Uy et al. 2001a) or about average male quality in the population (Dombrovsky and Perrin 1994). In this study, we also noted that females sample males over a prolonged period of several days. Furthermore we noted that the temporal distribution of courtship events in 2010 was bimodal (Figure 3.5), suggesting that peahens may also undertake two rounds of mate sampling, although other explanations are possible for this pattern, such as environmental factors, conspecific cueing (if females visit leks because they see other females doing so), or the possibility that females were sampling males at other leks that we did not observe during the dip in activity. There are other interesting similarities between peafowl and satin bowerbird mate sampling, based on the results of Uy et al. (2001a) and the results we report here: in both species, females appear to narrow their sampling effort, visiting fewer males, and spending more time being courted by each of those males, as they progress in the sampling process.
Spatial navigation is an interesting aspect of mate sampling behaviour that has thus far been ignored, except in studies examining the energetic costs of mate choice (e.g., Byers et al. 2005). Our results indicate that peahens tend to make substantially greater changes of course when travelling to a male that they visit, relative to other types of encounters. In other words, females tend to diverge more from their previous travel path when they head towards a male that they visit. This is consistent with the scenario that visits are planned, either because the females remember particular males and head towards them, or because the females are heading towards other long-range signals (such as calls) when they travel towards males. Other encounters including instances where females bypass or skip males may tend to be more haphazard.

We also found that females tend to travel substantially farther between encountered males as the breeding season progresses, consistent with the idea that females narrow the scope of their sampling with time. These results were based on limited data in which we assumed straight-line travel paths between encounters. High-resolution GPS data on female movements during mate sampling could provide more insight into the factors underlying these decisions, for instance through statistical analyses of travel paths to identify the precise location where females change direction on leks, asking which signals or behaviours are associated with these directional changes (e.g., Byrne et al. 2009; Joly and Zimmerman 2011). Such data could help reveal how females integrate information from a variety of sources, including male signals and other social cues, in real time during mate sampling. How does mate sampling vary with the spatial and temporal distribution of males and their signals in different populations and species? How
do males decide when to attempt copulation? How important is memory – for both males and females? The approach we used here of analyzing the outcome of repeated social interactions may be useful in future studies, especially where behaviours can be tracked remotely, to expand our knowledge of mate sampling and choice.

3.6 REFERENCES


Google Maps. 2012. [Los Angeles Arboretum, Arcadia, California] [Street map]. Retrieved from http://www.google.com/maps/ms?msid=213357681423656366778.0004a2787eaea05b363ab&msa=0&ll=34.144798,-118.051443&spn=0.001783,0.002411


CHAPTER 4. Sensory bias, sensory drive and eyespot colours in two peafowl species

4.1 ABSTRACT

Recently diverged species often show dramatic differences in their courtship signals, despite similarities in other traits. There are two species of peafowl in the genus *Pavo*, and their iridescent eyespots provide an interesting counter-example, since the sexually selected eyespot colours have been largely conserved despite drastic differences in other male colour traits. In this study, we use the train feather eyespots of blue (*Pavo cristatus*) and green (*P. muticus*) peafowl to test predictions of sensory bias and sensory drive, and we also investigate how male display behaviour alters the way females perceive the eyespot colours. We show that there are subtle but significant differences between the two species in the blue-green, purple-black and bronze regions of their eyespots. We then use models of avian colour vision to investigate how females would perceive male eyespot colours under different light conditions, and show that the subtle differences between the two peacock species may enhance different eyespot colour contrasts in their respective habitats, consistent with sensory drive. At the same time, we find that the blue-green colour on peacock eyespots achieves at- or near-maximal contrast with the bronze colour patch, relative to other hypothetical colours, consistent with a sensory bias explanation for the origin and evolutionary maintenance of these colours. In addition, we show that by orienting their iridescent trains at about 45° to the right of the sun’s azimuth, *P. cristatus* males maximize their eyespot colour contrasts for female observers, while potentially hindering females’ ability to discriminate between the colours of different males. Our results demonstrate that sensory biology has shaped multiple facets
of the peacock’s remarkable display, and may explain both the similarities and differences between *P. muticus* and *P. cristatus* eyespots.

4.2 INTRODUCTION

Theory and evidence indicate that signal evolution is often shaped by receiver perception. For instance, the theory of sensory drive states that signals will evolve for efficient transmission (Guilford and Dawkins 1991; Endler 1992; Endler 1993). In the case of the conspicuous colour traits used in courtship, this signal efficacy may be affected by a number of factors including the spectral properties of the colour signal, the visual background, the ambient light conditions, and the sensory physiology of the intended receivers (Endler 1993). When these details are known, it may be possible to predict the direction of evolution of colour traits (Endler 1992; e.g., Kemp et al. 2008; 2009). Indeed, across species, body colouration often varies with habitat light in a manner predicted to enhance colour signal detectability (e.g., Marchetti 1993; Endler and Théry 1996; Boughman 2001; McNaught and Owens 2002; Leal and Fleishman 2002; 2004; Heindl and Winkler 2003; Gomez and Théry 2004; Seehausen et al. 2008), and males may act in ways that further enhance their detectability, for instance by modifying the visual background (e.g., Uy and Endler 2004), their body posture (e.g., Hamilton 1965; Dakin and Montgomerie 2009; Olea et al. 2010; Bortolotti et al. 2011), or by presenting females with objects that have even greater colour contrast with the background (Endler et al. 2005).
Because signal efficacy depends on environmental conditions, sensory drive can lead to the divergence of signal traits in different habitats. When this process affects the signals involved in mate choice, it may contribute to reproductive isolation, and ultimately, speciation (Boughman 2001; 2002; Seehausen et al. 2008). It is generally thought that the process of sensory drive might explain why recently diverged species often show dramatic differences in their courtship signals, despite close similarity in other morphological and ecological traits (reviewed in Boughman 2002). For instance, African cichlid fishes exhibit striking diversification of male body colours, whereas cryptic females of related cichlid species can be difficult to distinguish. Research indicates that divergent female preferences for male colour traits in cichlids may have evolved as a consequence of changes in the visual system in different habitats (Maan et al. 2006; Seehausen et al. 2008). This finding indicates that sensory drive may have led to speciation; it is also consistent with the sensory bias model of sexual selection. Under this model, mate preferences result from biases towards certain signals or signal properties that are selected in other contexts, and that may be the result of properties of the sensory system (Ryan et al. 1990; Fuller et al. 2005). For instance, in Trinidadian guppies, females prefer males with orange-coloured spots, and both males and females are attracted to orange-coloured objects that resemble common food items in their stream habitats, implicating a pre-existing bias towards orange spots in the origin and exaggeration of this male colour trait (Rodd et al. 2002).

In practice, the processes of sensory bias and sensory drive may be difficult to distinguish (Fuller et al. 2005), and they need not be exclusive. In the case of colour signals, both
sensory bias and sensory drive predict that colour traits should evolve to optimally stimulate the female visual system. The main difference between the two theories is that sensory drive seeks to explain the divergence of signal traits depending on habitat conditions, whereas sensory bias seeks to explain the origin of preferences, and the exaggeration of the signal traits. Under sensory bias, signal preferences are ancestral, and they may potentially be conserved except where natural selection causes divergence in sensory function. Sensory drive can explain cases where signal traits diverge in different habitats, even when receiver perceptual abilities remain constant.

There is ample evidence that signals can diverge to match habitat characteristics, receiver perceptual sensitivities, or both (reviewed in Boughman 2002). What is less clear is how this may affect female fitness. For instance, selection for increased signal detectability under sensory bias or sensory drive could enhance females’ ability to find a mate efficiently, reducing the costs of mate searching and choice (Dawkins and Guildford 1996; Boughman 2002). Alternatively, it could have the opposite effect of increasing the costs of mate choice, by making it more difficult for females to discriminate among potential mates, or by causing females to be attracted to low quality males (as in chase-away sexual selection or sensory exploitation; Holland and Rice 1998; Arnqvist 2006).

4.2.1 Study system

The peacock’s iridescent eyespots provide an interesting contrast to cichlids and other taxa where the colour traits used in mate choice have rapidly diverged. In the genus *Pavo*, there are two closely related species, *P. cristatus* and *P. muticus*. Males of the two
species have drastically different body colours (Kannan and James 1998; Bergman 1980), yet despite these major differences, the eyespots that tip the majority of their train feathers are remarkably similar, especially the bronze, blue-green and purple-black regions (Figure 4.1; note also that the narrow bands of colour outside of the bronze region differ; see also Bergman 1980). In both blue (P. cristatus) and green (P. muticus) peafowl, these eyespots are displayed to females during courtship, and studies of P. cristatus indicate that the eyespot colours function in mate choice (Chapters 2 and 3). Courtship and mate choice have not been studied in P. muticus, yet it would be surprising if the eyespots of green peafowl are not also under strong sexual selection by female choice, given the broad similarities in courtship and the use of erect train displays in both species (Finn 1926; Bergman 1980).

Peafowl are an ideal system for testing hypotheses about the links between signal function, evolution and perception for several reasons. First, the visual sensitivity of P. cristatus has been characterized (Hart 2002), allowing us to estimate how conspecific females would perceive the peacock’s eyespot colours. For example, using models of avian colour vision, we recently found evidence that females’ perception of the blue-green eyespot colour influences mate choice in P. cristatus (Chapter 2). P. muticus visual physiology has not been characterized, but it is likely identical to that of P. cristatus, given the close relationship between the two species and the fact that avian photoreceptor sensitivities are generally highly conserved (Hart and Hunt 2007; e.g., Coyle et al. 2012).
FIGURE 4.1 Eyespots from (a-b) adult male blue (*Pavo cristatus*) and (c-d) green (*Pavo muticus*) peacocks. Each of the four eyespots shown in (b) and (d) is from a different male. Scale bars are 1 cm. The photo in (c) was taken by Jeremy Holden.
Second, the two *Pavo* species occupy habitats with different ambient light conditions: *P. cristatus* peacocks are distributed throughout semiarid regions of India and Sri Lanka, typically near cleared agricultural land, and they aggregate in open areas to court females (Ramesh and McGowan 2009; Harikrishnan et al. 2010). In contrast, *P. muticus* peafowl occupy more densely forested habitats in Southeast Asia (historically in parts of southern China, Vietnam, Laos, Cambodia, Thailand, Myanmar, Malaysia, and Indonesia; although now *P. muticus* is limited to isolated populations in Thailand, Vietnam and Indonesia; Bergman 1980; del Hoyo et al. 1994). Furthermore, surveys of *P. muticus* male breeding calls similar to those of *P. cristatus* indicate that courtship tends to occur in forested areas with approximately 30% canopy cover (Brickle 2002). The body colouration of female peafowl may also be indicative of different habitat preferences: *P. muticus* females have the same bright green body plumage as the males, whereas *P. cristatus* females have brown upperparts and a buff-coloured belly (Bergman 1980), possibly because this provides better camouflage in their open habitats where light and cover conditions are very different.

Another reason that makes peacock eyespots ideal for the study of signal design is that their colours are structural. In *P. muticus*, these colours are produced by highly organized crystal-like nanostructures of melanin rods connected by keratin within the feather barbules (Zi et al. 2003). The markedly different colours that can be seen in each eyespot are the result of slight changes in the dimensions and thickness of this interior nanostructure (Zi et al. 2003), without any change in the raw materials involved. The same nanostructures can also be observed in *P. cristatus* eyespots (Dakin and
Montgomerie, unpublished data). With this mechanism of colour production, considerable divergence in colouration is possible without requiring a drastic change in the raw materials or the developmental processes involved. Thus, one might expect that these colours should be relatively evolutionarily labile.

Fourth, these nanostructural colours are iridescent, so their signal properties are context-dependent. Different light angles, for example, can produce different perceptual effects for a stationary observer. We previously showed that *P. cristatus* males orient their erect trains at about 45° to the right of the sun’s azimuth, on average, during their courtship train-rattling displays (Dakin and Montgomerie 2009), and that male mating success is related to eyespot colours measured at this orientation (Chapter 2). This suggests that iridescence may be an important part of peafowl courtship, although it is not yet clear how male display orientation affects the transmission of these colour signals.

In this study, we use models of avian colour vision to investigate the eyespot colours of blue (*P. cristatus*) and green (*P. muticus*) peafowl. Our primary goal is to ask how sensory biology has influenced the evolution of the peacock’s eyespots. We start by comparing the eyespot colours of the two species. We then consider how females would perceive these eyespot colours under different light conditions. Specifically, we ask whether the two species have diverged in a manner that would enhance the impact of their eyespot colours in their respective habitats, as the theory of sensory drive would predict. We then use measured and simulated eyespot colours to test whether actual eyespot colours maximally stimulate the female sensory system, as one would predict.
under both sensory bias and sensory drive. Finally, we ask how light conditions during typical *P. cristatus* courtship displays affect signal perception, examining the impact of the colour contrasts in the eyespot pattern on female receivers, as well as females’ potential ability to discriminate between male colours during mate choice.

4.3 METHODS

4.3.1 Eyespot feather samples

We used 10 *P. cristatus* eyespot feathers and 10 *P. muticus* eyespot feathers to compare the two *Pavo* species. We obtained *P. muticus* eyespot feathers from breeders, 2 from a single male in 2010 (Countryside Exotics, Leamington, Canada) and 8 from another individual in 2011 (Brow Farm, Lancashire, UK). Previous work indicates that eyespot size, shape, and symmetry all vary systematically with feather location in the train ornament in *P. cristatus* (Dakin and Montgomerie 2011), and that the iridescence of the three main eyespot colours also varies with feather location (Dakin and Montgomerie, unpublished data), possibly due to differences in the tilt angle of feather barbules. We did not know where in the train our 10 *P. muticus* feathers had been located, but based on shape asymmetry, 4 of the 10 *P. muticus* feathers came from the left side of the train, and 4 came from the right, with 2 from at or very near the midline of the ornament.

Thus, to compare these eyespots with *P. cristatus* feathers from similar locations in the train ornament, we selected 10 feathers from the train of a single feral *P. cristatus* male (Los Angeles Arboretum, Arcadia, USA) that were an approximate match in terms of size and symmetry to the 10 *P. muticus* feathers. The *P. cristatus* eyespots we selected were
from two separate rows on the male’s train (with 4 from the left, 4 from the right, and 2 from the midline of that male’s ornament). We also used this set of 10 *P. cristatus* feathers to investigate the effect of illumination angle on *P. cristatus* eyespot colours.

### 4.3.2 Eyespot size and shape

To ensure that the samples described above were comparable, we measured eyespot size and symmetry on photographs using a flatbed scanner (hp Scanjet 7400c) to make a digital image of each feather (600 dpi bitmap, RGB colour), and then using the ‘lasso’ tool in Adobe Photoshop 10.0.1 (Adobe Systems 2008) to outline the bronze region on each feather and to determine the total area of pixels covered by the bronze, blue-green and purple-black regions as eyespot areal size (Figure 4.2). We converted this value to cm² using a scale on a standard grey card that we included in the background of every image. We quantified eyespot symmetry by using the ‘ruler’ tool in Adobe Photoshop to measure the maximum height in pixels of the left and right lobes of the purple-black patch on every eyespot, calculating an index of eyespot asymmetry as the difference between the height of the left and right lobes divided by the average of these two measurements.

### 4.3.4 Colour measurements

Feathers were stored in paper envelopes prior to taking colour measurements. To quantify eyespot colours across the bird-visible spectrum (300-700 nm), we used a USB4000-UV-VIS spectrometer (Ocean Optics, Dunedin, FL, USA) and an Ocean Optics DH-2000 Deuterium Tungsten Halogen light source (output 215-2000 nm) mounted in a
goniometer apparatus that allowed us to vary the illumination angle from 25-65° from the right of the feather surface, as viewed from the front. We measured reflectance at 90° to the feather surface, since *P. cristatus* females are positioned directly in front of males during the pre-copulatory train-rattling component of their courtship displays (Dakin and Montgomerie 2009). As in previous studies (e.g., Chapter 2), we used 400 µm optical fibers with collimating lenses for illumination (560 mm from the sample surface) and measurement (470 mm from the sample) of a spot about 2 mm in diameter, confirming the alignment of the two beams with a laser pointer. To exclude ambient light, all colour measurements were taken in a darkroom.

We measured the three main iridescent colour patches (bronze, blue-green and purple-black; Figure 4.2) on the right side of every eyespot, as viewed from the front, relative to a white standard made of Teflon™ tape layered to be the same thickness as the eyespot feathers. This ensured that the white standard could be mounted in our apparatus at the same distance from the illumination and measurement probes as the feather surface. Using SpectraSuite software (Ocean Optics 2009), we took the average of 10 scans of 100 ms integration time, with a boxcar smoothing function of 12 pixels. Every 15 minutes we recalibrated the white standard and took a new dark standard reading in a small black chamber that eliminated reflected light.
FIGURE 4.2 Eyespot size and colour measurements. (a) Areal size was measured by outlining the bronze region. Red vertical lines denote the measurements used to calculate eyespot symmetry. (b) Average reflectance spectra for the three main eyespot colours (bronze, blue-green and purple-black) in *Pavo cristatus* and *P. muticus*, using 10 feathers from each species, measured at a 45° illumination angle. Colour measurements were taken from the regions indicated in (a).
For all eyespot feathers, we measured each of the three main colour patches twice at a 45° illumination angle to simulate the average light angle during *P. cristatus* train-rattling displays (Dakin and Montgomerie 2009), re-mounting the samples in the apparatus between repeated measurements. This procedure yields highly repeatable measures of peacock eyespot colours (see Appendix C).

To examine the effect of male display orientation, we followed the same procedure described above, measuring feathers over a range of illumination angles from 25-65° from the right of the feather surface as viewed from the front (in 10° increments).

### 4.3.5 Tristimulus colour variables

We calculated tristimulus colour variables (hue, brightness and chroma) describing spectra shape using the program RCLR 0.9.33 (Montgomerie 2010). Following previous studies of *P. cristatus* (e.g., Loyau et al. 2007), we define hue as the wavelength of maximal reflectance, brightness as the total reflectance (or total area under the spectral curve), and chroma, or spectral purity, as the difference between the maximum and minimum reflectance divided by the average reflectance over the 300-700 nm range. We calculated these variables for each spectral measurement, taking the average of the two repeated measures of each feather.

### 4.3.6 Modeling colour perception

To estimate how females would perceive eyespot colours, we calculated the excitation or photon catch of retinal photoreceptors as the product of their sensitivity, the irradiance
spectrum, and the plumage colour spectrum over wavelengths from 300-700 nm. To do this, we used the average plumage colour spectrum calculated from our two repeat measurements of each colour patch. We used the four photoreceptor cone cell sensitivity curves reported for *P. cristatus* by Hart (2002; see Figure 7 therein), incorporating the filtering effects of cone cell oil droplets and the ocular media. We normalized these curves such that each curve had an area beneath it of 1 (Figure 4.3).

We modeled the perceived chromatic contrast between pairs of colours (or ΔS) following the receptor noise-limited model outlined by Vorobyev and Osorio (1998) using the R package pavo (Maia et al. 2012), taking account of relative photoreceptor densities on the peafowl retina as reported by Hart in 2002 (1, 1.9, 2.2, and 2.1 for the VS, SWS, MWS and LWS cones respectively), and assuming a Weber fraction of 0.05 for the UV-sensitive cone type (Håstad et al. 2005; Loyau et al. 2007) to calculate the noise for each photoreceptor type independently of the signal.

We used ΔS values to derive measures of discriminability and colour contrast of peacock eyespot colours. We defined “discriminability” as the ease with which similar colours might be discriminated (i.e., comparing the bronze on feather 1 with that of feather 2). To do this, we calculated pairwise ΔS values for a given patch against each of the corresponding patches on other feathers in a set for comparison. We then took the average of these ΔS values for a given patch as a measure of its “discriminability” against the comparison set. The greater the ΔS value, the more likely two colours will be perceived as being different when viewed simultaneously. In general, a value of ΔS > 3 is
considered to be a conservative threshold for a perceptible difference (Vorobyev et al.
1998), although it is common to use a threshold of $\Delta S > 1$ as a minimum level for a
discriminable difference (e.g., Morehouse and Rutowski 2010).

To estimate the perceived colour contrasts of eyespots, we calculated $\Delta S$ values for the
three possible pairwise chromatic contrasts within each eyespot feather (i.e., bronze vs.
blue-green, blue-green vs. purple-black, and purple-black vs. bronze; Figure 4.2).

We modeled eyespot colour perception using several irradiance spectra representing
different ambient light conditions taken from Vorobyev et al. (1998; see Figure 3a
therein), including: (i) the CIE reference spectrum for mid-morning/mid-afternoon
daylight (D55), (ii) CIE standard reference spectrum for noon daylight (D65), and (iii) a
spectrum of typical forest shade conditions. We normalized each irradiance spectrum to
have a maximum of 1 (Figure 4.3). P. cristatus males typically display in open habitats
during mid-morning and mid-afternoon, so the D55 spectrum should best represent light
conditions during P. cristatus courtship. The forest shade spectrum should more
accurately represent the light conditions during P. muticus courtship in forested habitats
(Brickle 2002).

We also modeled how females would perceive a range of simulated eyespot colours with
spectral shapes similar to those of P. cristatus and P. muticus eyespot colours (see
Appendix D for details).
FIGURE 4.3 (a) Spectral sensitivities of peafowl cone photoreceptors (from Hart 2002). Cone types are: VS = violet-sensitive; SWS = short wavelength-sensitive; MWS = medium wavelength-sensitive; and LWS = long wavelength-sensitive. (b) Irradiance spectra for mid-morning/mid-afternoon daylight (D55), noon daylight (D65), and forest shade light conditions.
4.3.7 Data analysis

Analyses were performed using JMP 10.0.0 and R 2.15.2 (R Core Team 2012). To evaluate the effect of different light conditions on how eyespot colours would be perceived, we calculated colour contrast and discriminability variables for a given sample of feathers in perceptual models, and then we repeated these calculations using different light conditions (either different irradiance spectra, or measurements taken at different light angles). The resulting variables thus represented repeated measures of the same feathers under different conditions. We therefore used mixed models to analyze these data, specifying feather as a random variable to account for repeated measures, using the R package nlme 3.1 to fit models with a Gaussian distribution of errors.

4.4 RESULTS

4.4.1 Eyespot size and shape

Areal size of the 20 *P. cristatus* and *P. muticus* eyespots used here ranged from 6.5-15.4 cm² (mean = 10.1, 95% CI = 9.1-11.1, n = 20), and the index of eyespot shape asymmetry ranged from –0.29-0.25 (mean = 0.016, 95% CI = –0.063-0.095). There was no difference between our *P. muticus* and *P. cristatus* samples in average eyespot areal size (*t*-test, t = 1.58, p = 0.13, df = 1,18) or asymmetry index (t = 0.46, p = 0.65); there was also no difference between the two samples in the absolute value of the asymmetry index (t = 1.46, 0.16).
4.4.2 Comparing eyespot colours of two species

Figure 4.2 shows the average reflectance spectra for the three main colour patches on *P. cristatus* and *P. muticus* eyespots measured at a 45° light angle. We found subtle but consistent differences between species in tristimulus colour variables for all three colour patches (Table 4.1). The bronze, blue-green and purple-black patches were all significantly brighter for *P. muticus*. There was no difference between species in the hue of the bronze patch. The blue-green patch has a significantly longer-wavelength hue in *P. muticus*, which to human observers makes the *P. muticus* blue-green patch appear slightly greener relative to the bluer blue-green of *P. cristatus* (Figure 4.1). The purple-black patch, on the other hand, has a significantly shorter-wavelength hue in *P. muticus* than it does in *P. cristatus*. As a result, females would perceive greater colour contrast between the blue-green and purple-black patches on *P. muticus* relative to *P. cristatus* (Table 4.1). At the same time, they would perceive less colour contrast between the purple-black and bronze patches on *P. muticus* relative to *P. cristatus*. We found no difference between species in perceived chromatic contrast of the bronze vs. blue-green patches.
Table 4.1 Tristimulus colour variables and colour contrasts of eyespot colours of blue (*Pavo cristatus*) and green (*P. muticus*) peacocks, measured at a 45° illumination angle. To determine perceived colour contrasts of the eyespot pattern, colour perception was modeled under standard noon daylight (D65) conditions. Means are given ± SE followed by the 95% confidence interval for 10 feathers from each species. Statistics are for $t$-tests comparing species (df = 1,18).

<table>
<thead>
<tr>
<th></th>
<th><em>P. cristatus</em> (n = 10)</th>
<th><em>P. muticus</em> (n = 10)</th>
<th>Test statistic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tristimulus colour variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bronze</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brightness (x 10^2)</td>
<td>48.82 ± 1.49</td>
<td>86.15 ± 3.03</td>
<td>$t = 11.06$</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>45.44-52.20</td>
<td>79.31-93.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chroma</td>
<td>1.97 ± 0.01</td>
<td>2.19 ± 0.02</td>
<td>$t = 9.57$</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>1.94-2.00</td>
<td>2.14-2.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hue (nm)</td>
<td>633.5 ± 1.5</td>
<td>636.7 ± 2.3</td>
<td>$t = 1.46$</td>
<td>$p = 0.16$</td>
</tr>
<tr>
<td></td>
<td>630.2-636.8</td>
<td>632.3-642.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blue-green</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brightness (x 10^2)</td>
<td>45.73 ± 3.19</td>
<td>95.96 ± 11.58</td>
<td>$t = 4.18$</td>
<td>$p = 0.0006$</td>
</tr>
<tr>
<td></td>
<td>38.52-52.95</td>
<td>69.77-122.15</td>
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<td></td>
</tr>
<tr>
<td>Chroma</td>
<td>3.91 ± 0.08</td>
<td>3.92 ± 0.11</td>
<td>$t = 0.04$</td>
<td>$p = 0.97$</td>
</tr>
<tr>
<td></td>
<td>3.74-4.08</td>
<td>3.68-4.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hue</td>
<td>492.1 ± 1.5</td>
<td>497.8 ± 1.7</td>
<td>$t = 2.47$</td>
<td>$p = 0.02$</td>
</tr>
<tr>
<td></td>
<td>488.7-495.5</td>
<td>493.9-501.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Purple-black</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brightness (x 10^2)</td>
<td>17.02 ± 0.31</td>
<td>23.64 ± 0.56</td>
<td>$t = 10.31$</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>16.32-17.71</td>
<td>22.62-24.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chroma</td>
<td>1.58 ± 0.05</td>
<td>1.34 ± 0.07</td>
<td>$t = 2.98$</td>
<td>$p = 0.008$</td>
</tr>
<tr>
<td></td>
<td>1.48-1.69</td>
<td>1.20-1.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hue (nm)</td>
<td>453.0 ± 0.9</td>
<td>444.5 ± 3.7</td>
<td>$t = 2.32$</td>
<td>$p = 0.03$</td>
</tr>
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<td></td>
<td>451.5-455.3</td>
<td>436.0-453.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Perceived colour contrast</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronze vs. blue-green</td>
<td>38.43 ± 0.82</td>
<td>40.23 ± 1.47</td>
<td>$t = 1.06$</td>
<td>$p = 0.30$</td>
</tr>
<tr>
<td></td>
<td>36.58-40.29</td>
<td>36.90-43.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue-green vs. purple-black</td>
<td>20.93 ± 0.30</td>
<td>25.33 ± 1.23</td>
<td>$t = 3.48$</td>
<td>$p = 0.003$</td>
</tr>
<tr>
<td></td>
<td>20.25-21.61</td>
<td>22.55-28.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple-black vs. bronze</td>
<td>24.41 ± 0.45</td>
<td>22.24 ± 0.48</td>
<td>$t = 3.29$</td>
<td>$p = 0.004$</td>
</tr>
<tr>
<td></td>
<td>23.39-25.43</td>
<td>21.16-23.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.4.3 Discriminability of species differences

To evaluate whether the differences between species might be detectable to female observers, we examined the discriminability of each *P. cristatus* colour patch against the corresponding set of *P. muticus* colours, under standard noon daylight (D65) conditions.

For the blue-green patch, discriminability ranged from 4.95-7.71 (mean = 6.43, 95% CI = 5.77-7.09), and for purple-black patch it ranged from 2.11-5.57 (mean = 3.63, 95% CI = 2.80-4.45, n = 10), suggesting that these species differences should generally be noticeable for females, at least when viewing stimuli simultaneously. The discriminability of *P. cristatus* bronze against *P. muticus* bronze ranged from 1.41-2.53 (mean = 1.88, 95% CI = 1.62-2.13), which is below the threshold of ΔS > 3, suggesting that there may be no consistent perceptible difference between the two species in the colour of this patch.

We also wanted to test whether the magnitude of the perceived differences between species would be greater than that within *P. cristatus*, so we compared the discriminability of *P. cristatus* vs. *P. muticus* colours with that of *P. cristatus* vs. *P. cristatus* colours. For all three eyespot colours, the discriminability of *P. cristatus* against *P. muticus* was greater than that of *P. cristatus* against *P. cristatus* (bronze b = 0.75, blue-green b = 2.38, purple-black b = 1.79; all p < 0.005, n = 20 measurements of 10 feathers). This indicates that on average, females may perceive greater colour differences between feather colours from the two species than they would between colours from two different
P. cristatus feathers, although as noted above the species difference in the bronze colour patch may not be perceptible.

4.4.4 Sensory drive and species differences

To test whether sensory drive might have contributed to the subtle differences in P. cristatus and P. muticus eyespot colours, we compared the colour contrasts of P. cristatus and P. muticus eyespots at 45° illumination under different ambient light conditions. We assumed that the mid-morning/mid-afternoon (D55) irradiance spectrum best represented typical light conditions during P. cristatus displays in open habitats, and that the forest shade irradiance spectrum best represented light conditions during P. muticus displays (Figure 4.3). Table 4.2 summarizes the results of models testing for the effect of these two different ambient light conditions, controlling for the difference between species. We first checked models including an interaction between ambient light and species, which we then eliminated since p-values for the interaction term were > 0.20 in every case.

Results in Table 4.2 indicate that the blue-green vs. purple-black contrast tends to be greater in forest shade, relative to open habitat light conditions. This contrast is also enhanced in P. muticus relative to P. cristatus. The purple-black vs. bronze contrast, on the other hand, tends to be greater in open habitat light conditions favoured by P. cristatus; this contrast is enhanced in P. cristatus relative to P. muticus.

The bronze vs. blue-green contrast is greater in open habitat light relative to forest shade; there was no difference between species in the magnitude of this contrast (Table 4.2). Of
the three pairwise contrasts in the eyespot pattern, the bronze vs. blue-green contrast is also the greatest in magnitude (Table 4.1).

4.4.5 Simulated hue shifts and eyespot colour contrasts

To better understand the effects of subtle shifts in eyespot colour hues, we simulated a range of eyespot colours and modeled how they would be perceived (see Appendix D for details). Our results indicate that the blue-green colour of actual peacock eyespots in both species achieves at- or near-maximum contrast with the bronze colour patch, and that the divergence in eyespot hues between *P. cristatus* and *P. muticus* may be the result of selection for increased eyespot colour contrasts in their respective habitats.
Table 4.2 Effect of ambient light on the colour contrasts of eyespot feathers from two peacock species, comparing mid-morning/mid-afternoon (D55) daylight and forest shade conditions. Statistics are given for mixed models testing the effects of species and irradiance spectrum (n = 40 measurements of 20 feathers, 10 from *P. cristatus* and 10 from *P. muticus*). Interaction terms were removed from all models as p > 0.20 in every case.

<table>
<thead>
<tr>
<th>Response</th>
<th>Predictors</th>
<th>Estimate</th>
<th>SE</th>
<th>F</th>
<th>p</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronze vs. blue-green</td>
<td>species (<em>P. muticus</em>)</td>
<td>1.92</td>
<td>1.65</td>
<td>1.38</td>
<td>0.25</td>
<td>1,18</td>
</tr>
<tr>
<td></td>
<td>light (forest shade)</td>
<td>−1.21</td>
<td>0.04</td>
<td>1056</td>
<td>&lt; 0.0001</td>
<td>1,19</td>
</tr>
<tr>
<td>Blue-green vs. purple-black</td>
<td>species (<em>P. muticus</em>)</td>
<td>4.41</td>
<td>1.27</td>
<td>12.4</td>
<td>0.02</td>
<td>1,18</td>
</tr>
<tr>
<td></td>
<td>light (forest shade)</td>
<td>0.05</td>
<td>0.01</td>
<td>20.0</td>
<td>0.0002</td>
<td>1,19</td>
</tr>
<tr>
<td>Purple-black vs. bronze</td>
<td>species (<em>P. muticus</em>)</td>
<td>−2.09</td>
<td>0.64</td>
<td>11.0</td>
<td>0.004</td>
<td>1,18</td>
</tr>
<tr>
<td></td>
<td>light (forest shade)</td>
<td>−1.15</td>
<td>0.05</td>
<td>632</td>
<td>&lt; 0.0001</td>
<td>1,19</td>
</tr>
</tbody>
</table>
4.4.6 Effect of light conditions during courtship on P. cristatus eyespot colours

4.4.6.1 Illumination angle

The brightness, chroma and hue of iridescent P. cristatus eyespot colours all change with illumination angle \( \theta \) (Figure 4.4), and thus would change with male display orientation. To investigate how this would affect female perception of the eyespot colours, we used polynomial models to test the effects of both \( \theta \) and \( \theta^2 \). This allowed us to model the curvilinear change in perceived colour contrasts with illumination angle (Figure 4.5), and to test whether colour variables were maximized at the 45° angle observed during P. cristatus displays (Dakin and Montgomerie 2009).

We first examined whether male display orientation might influence the ability of females to perceive variation among males, by testing whether illumination angle affected the discriminability of P. cristatus eyespot colours against other P. cristatus colours from the corresponding patch. Results are summarized in Table 4.3. Under standard daylight conditions, illumination angle did not affect discriminability of the blue-green or purple-black patches in any consistent way. For the bronze patch, discriminability was somewhat diminished at angles closer to 45°, but not significantly so. Overall, we found no evidence that the 45° light angle maximizes discriminability of any of the three main eyespot colours.
FIGURE 4.4 Tristimulus colour variables (a) brightness, (b) chroma, and (c) hue of *P. cristatus* eyespot colours in relation to illumination angle. Lines connect measurements from the same feather (*n* = 10 feathers).
FIGURE 4.5 Eyespot colour contrasts in relation to illumination angle, showing (a) bronze vs. blue-green, (b) blue-green vs. purple-black, and (c) purple-black vs. bronze. Perceived contrasts were modeled under noon daylight (D65) conditions. Regression lines are calculated from polynomial mixed models (see Table 4.3).
The effect of illumination angle on the colour contrasts of the *P. cristatus* eyespots is also shown in Table 4.3. All three pairwise chromatic contrasts changed with illumination angle in a curvilinear fashion (Figure 4.5). The mean angle of maximum contrast was 46° for blue-green vs. bronze, 52° for bronze vs. purple-black, and 54° for blue-green vs. purple-black (from the right of the feather surface, as viewed from the front). This suggests that males can achieve maximum eyespot colour contrast by orienting their trains at about 35-45° to the right of the sun’s azimuth.

**4.4.6.2 Time of day**

We investigated how ambient light at different times of day might influence female perception of the eyespot colours by comparing the discriminability and colour contrasts of *P. cristatus* eyespot colours under D55 and D65 light conditions. We first examined discriminability of *P. cristatus* eyespot colours against other *P. cristatus* colours from the corresponding patch (Table 4.4). Results suggest that variation in all three colours should be more readily discriminated under noon daylight conditions (D65), relative to mid-morning/mid-afternoon conditions (D55). As well, all three pairwise chromatic contrasts in the eyespot pattern are greater under noon daylight conditions (D65), relative to mid-morning/mid-afternoon light conditions (D55). Together, these results suggest that light conditions at peak lekking times for *P. cristatus* (i.e., earlier in the morning and later in the afternoon) may diminish both the discriminability and the colour contrast of *P. cristatus* eyespot colours, at least relative to typical light conditions at mid-day.
Table 4.3 Discriminability and colour contrasts of *P. cristatus* eyespot colours in relation to illumination angle under standard noon daylight conditions. Illumination angle (θ) ranged from 25° to 65° in 10° increments. Statistics are given for mixed models testing the effect of θ and θ² (n = 50 measurements of 10 feathers from a single *P. cristatus* male).

<table>
<thead>
<tr>
<th>Response</th>
<th>Illumination angle predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Discriminability Estimate</td>
</tr>
<tr>
<td></td>
<td>θ</td>
</tr>
<tr>
<td></td>
<td>θ²</td>
</tr>
<tr>
<td></td>
<td>Blue-green</td>
</tr>
<tr>
<td></td>
<td>θ</td>
</tr>
<tr>
<td></td>
<td>θ²</td>
</tr>
<tr>
<td></td>
<td>Purple-black</td>
</tr>
<tr>
<td></td>
<td>θ</td>
</tr>
<tr>
<td></td>
<td>θ²</td>
</tr>
</tbody>
</table>

Colour contrasts

|                      | Bronz vs. blue-green        |    | 0.56 | 0.09 | 39.3 | < 0.0001  |
|                      | θ²                          | -0.006 | 0.001 | 36.9 | < 0.0001  |
|                      | Blue-green vs. purple-black | θ   | 0.28  | 0.07  | 14.99 | < 0.0001  |
|                      | θ²                          | -0.002 | 0.0008 | 9.03 | 0.005 |
|                      | Purple-black vs. bronze     | θ   | 0.36  | 0.04  | 85.0  | < 0.0001  |
|                      | θ²                          | -0.003 | 0.0004 | 64.4 | < 0.0001  |
Table 4.4 Discriminability and colour contrasts of *P. cristatus* eyespot colours in relation to time of day, comparing mid-morning (D55) light conditions to standard noon daylight (D65) conditions. Statistics are given for mixed models testing the effect of the irradiance spectrum (n = 20 measurements of 10 feathers from a single *P. cristatus* male).

<table>
<thead>
<tr>
<th>Response</th>
<th>Irradiance predictor</th>
<th>Estimate</th>
<th>SE</th>
<th>F</th>
<th>p</th>
<th>df</th>
</tr>
</thead>
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<td>Discriminability</td>
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<tr>
<td>Bronze light (D55)</td>
<td>−0.04</td>
<td>0.003</td>
<td>183</td>
<td>&lt; 0.0001</td>
<td>1,9</td>
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<td>Blue-green light (D55)</td>
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<td>0.007</td>
<td>9.42</td>
<td>0.01</td>
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<td>Purple-black light (D55)</td>
<td>−0.009</td>
<td>0.002</td>
<td>17.2</td>
<td>0.003</td>
<td>1,9</td>
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<tr>
<td>Colour contrast</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bronze vs. blue-green</td>
<td>light (D55)</td>
<td>−0.19</td>
<td>0.02</td>
<td>82.0</td>
<td>&lt; 0.0001</td>
<td>1,9</td>
</tr>
<tr>
<td>Blue-green vs. purple-black</td>
<td>light (D55)</td>
<td>−0.07</td>
<td>0.006</td>
<td>172</td>
<td>&lt; 0.0001</td>
<td>1,9</td>
</tr>
<tr>
<td>Purple-black vs. bronze</td>
<td>light (D55)</td>
<td>−0.45</td>
<td>0.01</td>
<td>1387</td>
<td>&lt; 0.0001</td>
<td>1,9</td>
</tr>
</tbody>
</table>
4.5 DISCUSSION

Why are the eyespot colours of blue and green peacocks so remarkably similar, despite drastic differences in male body colour? And why are there subtle differences between these two species in the blue-green and purple-black regions of their eyespots? Our results point to potential explanations for both questions.

First, our results indicate that the subtle divergence between species in eyespot colours is likely due to sensory drive. Specifically, in *P. muticus*, the contrast between the blue-green and purple-black patches is enhanced relative to *P. cristatus*, and this contrast also tends to be greater in the forested habitat preferred by *P. muticus* (Table 4.2). *P. cristatus* eyespots, on the other hand, have enhanced purple-black vs. bronze contrast, which is greater in their open habitats. Our colour simulations also indicate that subtle shifts in the blue-green hue of peacock eyespots may have different effects in different habitats. In open habitat light, the bluer *P. cristatus* blue-green may enhance the blue-green vs. bronze contrast at the expense of the blue-green contrast with purple-black (Figure D2 in Appendix D). In forest shade conditions, this trade-off is not as strong, and maximal blue-green vs. bronze contrast can be achieved over a much wider range of blue-green hues (Figure D3 in Appendix D). As a result, *P. muticus* can achieve greater blue-green vs. purple-black contrast while still maintaining maximal blue-green vs. bronze contrast. Thus, *Pavo* eyespots appear to have diverged to enhance eyespot colour contrast in different habitats, consistent with sensory drive.
Sensory drive and selection for signal efficacy might also explain why *P. muticus* eyespots are considerably brighter, with greater chroma, or spectral purity, than *P. cristatus* eyespots; enhanced brightness could enhance signal transmission in darker forested habitats for *P. muticus* (Endler 1992). Consistent with this, a negative relation between plumage brightness and the intensity of habitat light has been reported in *Phylloscopus* warblers (Marchetti 1993), although in some other bird groups, the opposite trend has been found (i.e., brighter plumage in more open habitats; McNaught and Owens 2002; Gomez and Théry 2004).

Our results support a sensory bias model of sexual selection as the explanation for the close similarity between species in their eyespot colours. Under the sensory bias model, females might be predicted to prefer colour combinations that produce maximal perceived contrast as a result of ancestral, conserved features of the visual system. We previously found that *P. cristatus* peacocks with greater eyespot colour contrast achieve greater mating success, suggesting that females prefer certain males based on how they perceive this trait (Chapter 2). In addition, our simulations of a range of eyespot colours suggest that actual peacock eyespots may achieve a balance of high contrast between the three main eyespot colours. Furthermore, our simulations also show that the blue-green hue produces at- or near-maximal contrast with the bronze colour patch in both species (Appendix D). All three of these findings are consistent with the sensory bias model; they are also consistent with a sensory drive scenario for the evolution of the eyespot hues. However, the fact that this particular combination of colours has been maintained by two species in different habitats – despite the potential for evolutionary change, as indicated
by divergence in other male traits including the less prominent outermost eyespot colours (Figure 4.1) – suggests to us that females might have a general bias towards this particular combination of colours, independent of habitat light.

Our results testing the effect of illumination angle point to an intriguing conflict between the sexes over courtship signaling. We showed that in *P. cristatus*, the orientation at which males typically display should increase the colour contrast of the eyespots for female observers (Figure 4.5), a trait that females prefer (Chapters 2 and 3). Our results also indicate that when males orient their train displays at 45° to the light, it should not improve females’ ability to perceive subtle variations between different feathers. Thus, males display their trains in a manner that may influence female mate choice – and these behaviours might hinder females’ ability to evaluate males accurately on the basis of their colour traits. At the same time, we also found that light conditions at mid-morning when *P. cristatus* males typically display would not enhance the impact of the eyespot colour contrasts, or females’ ability to perceive variation between them.

There are several limitations here that should be noted. First, we inferred females’ ability to discriminate between sequentially-viewed stimuli, as they would be experienced during mate choice, using ΔS values from models of avian colour perception. Ours is not the first study to have applied these models in this context (e.g., see also Morehouse and Rutowski 2010). These models were intended to represent the discrimination of simultaneous stimuli, and while they have been shown to accurately predict birds’ threshold spectral sensitivities in behavioural tests (Vorobyev and Osorio 1998), as well
as their efficiency at detecting food items (Maddocks et al. 2001), these models do not incorporate other higher-level processes that may influence the classification or comparison of sequentially-viewed stimuli. So far, these higher-level processes are not well understood, especially in the context of signaling and mate choice behaviour. Thus, given that the predictions of photon catch-based models correlate well with results from behavioural studies of discrimination of simultaneous stimuli (Vorobyev and Osorio 1998) and foraging preferences (Maddocks et al. 2001) in birds, it seems reasonable to assume that $\Delta S$ on its own should correlate with the ease of discriminating or classifying sequentially-viewed stimuli (see also Morehouse and Rutowski 2010).

Another limitation of this study is that photoreceptor sensitivities have only been characterized for male $P.\ cristatus$ peafowl, and we assumed here that the female visual system is identical. We also assumed that $P.\ cristatus$ vision is representative of both $Pavo$ species. These assumptions should be reasonable, given that these species are so closely related, and that photoreceptor sensitivities tend to be highly conserved in birds (Hart and Hunt 2007; e.g., Coyle et al. 2012). Nevertheless, it is possible that the two $Pavo$ species diverge in photoreceptor sensitivities, and if so, our analysis of the impact of $P.\ muticus$ eyespot colour contrasts on conspecific females would have to be re-evaluated – though it may be even greater than we estimated here.

Another important limitation is our use of a small set of feathers from only 3 individual males as exemplars to compare the two species. We chose this small sample because it allowed us to match feathers in terms of eyespot size and shape, and thus location in the
train ornament. This is an important control given that eyespot iridescence varies with feather location in the train ornament in peafowl. Indeed, we have noted that in *P. cristatus*, eyespots from opposite sides of the train can have hue differences as great as the difference between species reported here (Dakin and Montgomerie, unpublished data). Thus, it is crucial to control for feather location when comparing eyespot colours, and we suspect that such specialization in terms of location on the body may be important in other iridescent animal signals as well.

It is interesting that the two peacock species do not differ in the bronze patch hue. The bronze patch is also distinct from the other two main eyespot colours in that it has a bimodal spectrum produced by a slightly different nanostructure (a rectangular lattice, versus square lattices for the blue-green and purple-black patches; Zi et al. 2003). In addition, the bronze nanostructure is only ~4 layers thick, versus ~9-12 layers for the other two main eyespot colours (Zi et al. 2003). Perhaps the bronze structure may be developmentally constrained in some way that the other colours are not – although the fact that there is substantial variation in bronze hue in both species would suggest otherwise (Table 4.1). Another possibility is that the bronze colour amplifies the impact of the eyespot pattern by contrasting with blue-green, but that shifts in the bronze hue have little to no impact on female observers, resulting in little direct selection on the bronze colour (Hasson 1989). Consistent with this scenario, our simulations suggest that in open habitat light at least, shifts in the bronze hue may have little to no effect on its contrast with the highly chromatic blue-green patch (Figure D2 in Appendix D), although
this does not appear to be the case under forest shade conditions (Figure D3 in Appendix D).

In summary, our findings illustrate that sensory biology may explain both the divergence and similarity between two peacock species. Behavioural experiments are needed to confirm our results, for instance by testing how females respond to different colour combinations under different light conditions. Such experiments could also be used to test our hypothesis that male display orientation may enhance eyespot colour contrast while making it more difficult for females to choose an appropriate mate. It would be also interesting to know how males develop this orientation behaviour: is it an instinctual response, or do peacocks learn to present their iridescent trains for optimal effect, perhaps by observing the effect of their courtship displays on females? Lastly, psychophysical experiments and studies of the genetics and development of eyespot colours are also needed to better understand the sources of variation in these colours, and how multiple colours in the eyespot pattern evolve in concert. On the whole, the peacock’s eyespots are a promising system to explore the influence of sensory biology, development, and sexual conflict on signal design.

4.6 REFERENCES


Montgomerie R. 2010. RCLR 0.9.33. Queen’s University, Kingston, Canada. (available at http://post.queensu.ca/~mont/color/analyze.html)


CHAPTER 5. General discussion

5.1 OVERVIEW

Females will often go to great lengths to seek out encounters with males: pronghorn antelope (*Antilocapra americana*) roam for several additional kilometers per day to visit males during the annual rut (Byers et al. 2005), and satin bowerbirds (*Ptilonorhynchus violaceus*) spend up to two weeks visiting and revisiting different males before mating (Uy et al. 2001). In many cases, we know something about what these females are doing – evaluating males on the basis of certain signals and traits – but much less about how and why (Andersson 1994). How do memory, perception and decision-making mechanisms guide female choice? What are the sources of selection on and evolutionary history of these mechanisms?

Conspicuous colours are thought to influence mate choice and other social interactions in a variety of taxa (Andersson 1994). In the past two decades, models have been developed that allow us to estimate how animals perceive these colour signals (Montgomerie 2006). These models are now being applied to study how animals make choices in the context of courtship (e.g., Loyau et al. 2007; Maan and Cummings 2009; Morehouse and Rutowski 2010) and parental care (e.g., Spottiswoode and Stevens 2011).

In this dissertation, I applied these models to the peacock’s multi-coloured eyespots. My goal was to evaluate male colour signals as females would perceive them during
courtship, and to use these measurements to better understand females’ choice decisions, as well as signal function and evolution.

5.2 SUMMARY AND IMPLICATIONS

Figure 5.1 summarizes the results of the data chapters in this thesis and how they relate to one another. In Chapter 2, I used both observation and experiment to show that the colouration of the eyespot pattern strongly influences male mating success. I measured male eyespot colours as females would perceive them during courtship, and found a strong relationship between eyespot colour and male mating success. The results of Chapter 2 demonstrate that we can better understand selection on colour signals by considering the context in which those colours are displayed.

I then asked in Chapter 3 how females use eyespot colours and other features of the train ornament when deciding to visit males for courtship, and how this might influence the process of gathering information during mate choice. I found evidence that females remember the males they have visited in the past, since a female’s prior experience with a given male affects how she subsequently responds to him. I also found evidence that prior experience affects how females use male signals when deciding who to visit. For instance, females only skip males with non-preferred eyespot colours after they have already viewed their displays at close range, suggesting that they may use different cues when deciding to visit a male for the first time.
Figure 5.1 Diagram summarizing the results of this thesis.
The fact that females might remember the males they visit is not surprising: recent evidence has proven that birds often have remarkable memories, with the ability to remember specific group mates (e.g., Godard 1991; Boeckle and Bugnyar 2012) and even specific human faces after a single encounter (e.g., Levey et al. 2009; Marzluff et al. 2010). However, memory for individuals has not yet been examined in the context of courtship signaling and mate sampling, even though it may be a key feature of comparative mate choice.

In Chapter 4, I investigated how light conditions influence female perception of the eyespots. My approach was based on previous chapters showing that females prefer male colour traits (Chapters 2-3), as well as a previous study showing that *P. cristatus* males perform their courtship displays at roughly 45° to the sun’s azimuth (Dakin and Montgomerie 2009). In Chapter 4, I found evidence that eyespot colour contrast – a trait that females may prefer – is maximized at the 45° light angle that males use during display. In addition, my results suggest that these light conditions should not facilitate the ability of females to discriminate between the colours of different males.

I also compared the eyespot colours of two peafowl species in Chapter 4. Blue (*Pavo cristatus*) and green (*P. muticus*) peacocks have very similar eyespot colours, although they differ dramatically in their other plumage colour traits. I found evidence that the subtle differences between the eyespot colours of the two species may be due to differences in habitat light conditions. At the same time, my results suggest that the general maintenance of the eyespot colours might be due to selection for enhanced
chromatic contrast, consistent with the idea that sensory biology has played a role in shaping this trait.

The results of Chapter 4 indicate that perceptual effects can influence both the morphological basis of a nanostructural colour signal and the display behaviours used when presenting that signal to its intended audience. Together with the results of Chapters 2-3, these results also point to a potential source of conflict between the sexes. Specifically, females appear to invest considerable effort into visiting the most stimulating males, spending several days viewing males and apparently keeping track of the males they visit (Chapter 3). Males, on the other hand, display their trains in a manner that alters the way females perceive their eyespot colours (Chapter 4), potentially enhancing their attractiveness and influencing female choice (Chapters 2-3). To my knowledge, this is the first study to establish a link between presentation behaviour, perception and choice in this way (Figure 5.1), consistent with the idea that sexual conflict might play out in signal perception and evaluation (Holland and Rice 1998; Arnvist 2006; Chapman 2006).

Chapters 2 and 3 also have implications for our understanding of the function of multiple ornamental traits: the results of Chapter 3 suggest that females may use different aspects of the train ornament at different times when deciding to visit males for courtship, as has been suggested for other species with complex courtship displays (e.g., Robson et al. 2005). Thus, multiple features of a complex display can potentially perform similar functions, with the use of different features depending on the context. At the same time,
the results of Chapter 2 indicate that some aspects of the iridescent eyespot colour pattern may not enter into the mate choice decision. More work is needed to understand why the other colours exist when female choice is primarily based on the highly iridescent blue-green part of the eyespots, but an intriguing possibility is that the other colours enhance the effect of the blue-green through contrast effects (Chapter 4 and Appendix D).

5.3 FUTURE DIRECTIONS

The peacock may be a useful model for further research on courtship signaling and mate choice. In the following sections, I outline directions for future work on this subject, as well as other questions relating to the findings I presented here.

5.3.1 Eyespots and peafowl

The fact that peafowl are amenable to captive breeding suggests that it should be possible to test how females respond to a variety of stimuli under controlled conditions. This could be a promising approach for investigating the causes and consequences of female perceptual bias for the eyespot pattern. As a relatively simple pattern, the eyespot may be ideally suited for creating a range of artificial stimuli. How do females respond to variations in terms of eyespot shape, colour contrast, and arrangement of the three main colours? How well do they discriminate differences in the blue-green colour, and how do they respond to dynamic iridescent effects? If artificial stimuli are used, a major challenge would be to produce colours that stimulate the photoreceptors of birds in a manner that mimics the effect of actual eyespot feathers. This is because our methods of rendering colour in print and on screen rely on colour mixing with three primary colours
based on human trichromatic colour vision. Colour rendering methods for human observers also do not incorporate wavelengths in the UV range, which birds can perceive. For now, it may be impossible to design artificial stimuli that reproduce the effect of the peacock’s eyespots on avian observers exactly (Fleishman et al. 1998). Nevertheless, given established photon catch-based models of peafowl vision, it should at least be possible to determine how well different stimuli mimic the eyespot colours, as well as the effect of variations of those stimuli.

Another logical follow-up to this work would be to study male signals in situations where females are presented with live, displaying males under controlled artificial lighting. These methods could be used to compare female preference for *Pavo cristatus* and *P. muticus* males – one could test whether females prefer *P. cristatus* males under open habitat light conditions versus forest shade, and vice versa for *P. muticus* males. Additionally, by controlling the angle and intensity of artificial lighting, one could directly test how light influences female choice decisions. Can males be made to appear more attractive to females using directed artificial lighting? One simple technique that may be especially useful for this may be to keep males and females in adjacent enclosures with “peepholes” that limit females to viewing one male at a time, and that allow viewing time to be easily quantified (e.g., Bird and Emery 2008; Grodzinski et al. 2012). This set-up might also be useful in further studies of the importance of memory during mate assessment and choice.
Recently, an eye-tracking device for peafowl has also been developed and used to study how peahens view males during their courtship displays (Jessica Yorzinski et al., unpublished data presented at the 47th Annual Meeting of the Animal Behaviour Society in 2010). It would be interesting to see how females respond to a variety of artificial eyespot stimuli using this device – comparing, for instance, different colour contrast effects and different arrays of eyespots. Eventually, it may even be possible to locate the neural basis for females’ responses to the eyespots, as neurophysiological techniques can now be used to record activity from specific brain regions in singing birds (e.g., Fortune et al. 2011) and running mice (Harvey et al. 2012). Currently, it should also be possible to explore this using positron emission tomography (PET) imaging on females anesthetized shortly after exposure to a stimulus of interest, as Marzluff et al. (2012) have recently done to investigate individual recognition in crows (*Corvus brachyrhynchos*). It would be fascinating to apply these imaging techniques to courtship – for instance contrasting the effect of more or less attractive males, or artificial stimuli. These methods could also be used to examine females’ responses to familiar and unfamiliar males, providing additional evidence for the role of memory in mate choice.

### 5.3.2 Signal enhancement and learning

In Chapter 4, I found evidence that peacocks display in a way that enhances the attractiveness of their eyespots for female observers. Other birds are also known to modulate their courtship signals, adjusting the intensity or frequency of their displays depending on the social context (e.g., Patricelli et al. 2006; Smith et al. 2011). We know almost nothing about how these behaviourally plastic displays develop, but one
possibility is that they are learned. In the 1980s, for example, West and King (1988) discovered that young male cowbirds (*Molothrus ater*) learn to refine their song repertoires by attending to subtle movements of females that indicate which songs the females prefer. Such learning through reinforcement may be universal in animals (Shettleworth 2010), but so far the consequences of learning for mate choice and sexual conflict have not been thoroughly explored. For example, to what extent does learning contribute to the conflict over mate choice proposed by Holland and Rice (1998)?

In peafowl, there is a great deal of variation between males in extent to which they interact with females (Figure 2.5), and hence their opportunity to learn from reinforcement during those interactions. This might also contribute to the maintenance of variation in male mating success (i.e., the lek paradox; reviewed in Kotiaho et al. 2008). Learning opportunity could also explain the origins and maintenance of deception in other signals, such as the deceptive alarm calls documented in some species (e.g., Bro-Jørgensen and Pangle 2010; Flower 2011). The potentially important role of learning opportunity as a mechanism for maintaining the rarity of deceptive signals has not yet been considered.

The peacock’s train display would be an interesting case for investigating these ideas: chicks (male and female) will raise their tails shortly after hatching, and juvenile males frequently display to females and other males before developing the full train in their fourth year. To what extent does reinforcement and practice shape male display behaviour, and what are the long-term consequences for courtship and mate choice? A
promising way to investigate this might be to allow young, inexperienced males to court females in captivity, using an apparatus that allows the experimenter to control whether the females are able to see and respond to the males’ actions (e.g., using one-way glass). If peafowl will respond to an image of another bird on a video screen, a closed-loop teleprompter system could also be used to allow more precise control over the exchange of visual cues between males and females, as has recently been done with *Columba livia* pigeons (Ware 2011).

5.3.3 *Travel paths and mate choice*

The pattern of female movements during mate choice is another area that is ripe for future study, especially given recent advances in geolocation technology that now allow us to track the travel paths of individual animals with extraordinary precision. In the last few years, these tracking methods have led to new insights into a variety of foraging, dispersal, and migration behaviours (e.g., Mabry and Stamps 2008; Byrne et al. 2009; Joly and Zimmerman 2011; Lanzone et al. 2012). With more detailed data on female travel paths during courtship, especially where these data can be collected automatically from a large number of individuals, and used to infer their social interactions, signals, and responses in real-time (e.g., Mennill et al. 2012; Rutz et al. 2012), a more complete picture of mate choice should emerge. Such detailed data could also be used to better address the role of memory and comparison during courtship and mate choice.
5.3.4 Comparisons with other species

Some birds and mammals encounter different males over an extended period of days or even weeks of courtship (e.g., Uy et al. 2001; Byers et al. 2005), whereas in other species females may copulate within a few hours of their first encounter with a male (e.g., Ryan et al. 1981; Ryan 1983). How do the cognitive mechanisms of choice vary with the spatial and temporal distribution of male encounters? In birds at least, there are a number of distantly related polygynous species that, like peafowl, invest a considerable amount of time into mate sampling, as they tend to pay repeat visits to males over several days (e.g., great reed warblers *Acrocephalus arundinaceus*, Bensch and Hasselquist 1992; satin bowerbirds *Ptilonorhynchus violaceus*, Uy et al. 2001). Túngara frogs (*Engystomops pustulosus*) are also polygynous, but mate sampling may be especially costly because predatory bats are attracted to the same features of male calls that females prefer (Ryan et al. 1982; Akre et al. 2011). Thus, natural selection may act against túngara females that invest time to accurately compare many different males, even if they are able to do so.

5.3.5 Choice in other contexts

The study of mate choice may be relevant to our understanding of animal choice and decision-making mechanisms in general. Historically, most of the research on the cognitive mechanisms of choice in animals has focused on foraging decisions (see Shettleworth 2010), but as several authors have pointed out, adaptations for foraging decisions may be co-opted for mate choice (e.g., Ryan et al. 2009) or vice versa (e.g., Kacelnik et al. 2011). To what extent are choice mechanisms common across these different contexts, and are similarities based on shared neural and physiological
mechanisms? Do other types of social choice, such as parental investment, draw on the same mechanisms as mate choice (Lyon and Montgomerie 2012)? In psychology, there is extensive evidence that humans often apply certain heuristics or shortcuts that may improve the efficiency of choice, and some of these heuristics may apply to animal foraging decisions as well (Bateson and Healy 2005). Do they also apply to social choice in animals? It would be interesting to see how humans respond to decision-making tasks analogous to those faced by females during mate choice (e.g., Sullivan et al. 1995), as this could potentially lead to further insights into the mechanisms that animals may (or may not) use.

5.4 CONCLUSIONS

Choosing a mate is a decision of fundamental importance in an animal’s life. Despite several decades of research on mate choice, we are only just beginning to understand the mechanisms underlying these decisions, and how these mechanisms influence behaviours on both sides of the courtship signaling exchange. In this thesis, I showed that the perceptual effects of the peacock’s eyespot colours might alter male appearance in a way that enhances male attractiveness. Further experimental study of the cognitive mechanisms involved, and their consequences in peafowl courtship interactions, will help move us towards a better understanding of signaling, mate choice and sexual selection.

5.5 REFERENCES


Ware ELR. 2011. Interactive behaviour in pigeons: visual display interactions as a model for visual communication. PhD thesis, Queen's University, Kingston, ON.

APPENDIX A

SUPPLEMENTARY MATERIALS TO CHAPTER 2

In this supplement, we provide additional details of the methodology used in Chapter 2, and a summary of the model sets generated using the Information-Theoretic approach to model evaluation.

A.1 Additional methodological details

The peacock’s eyespots vary systematically in both their degree of symmetry (Dakin and Montgomerie 2011) and their iridescence (R Dakin, unpublished data) according to their location of feather insertion on the uropygium (and thus the position of the eyespot in the erect ornament). For this reason, we needed a method to ensure that we measured the eyespot on the same feather from each male’s train, so we measured eyespot symmetry and chose the most symmetrical eyespot for colour analysis.

To measure eyespot symmetry, we first used a flatbed scanner (hp Scanjet 7400c) to make a digital image of all the feathers sampled from each male (600 dpi bitmap, RGB colour). We then used the ‘ruler’ tool in Adobe Photoshop 10.0.1 (Adobe Systems, 2008) to measure the maximum height in pixels of the left and right lobes of the purple-black patch on every feather. We calculated a relative index of eyespot asymmetry as the absolute value of the difference between these two heights divided by their average, selecting the single most symmetrical feather for further analysis, to ensure that we obtained the feather that would be closest to the midline on the train ornament (Dakin and Montgomerie 2011; R Dakin, unpublished data).
A.2 Tetrahedral colour space model

Tetrahedral colour space models (Goldsmith 1990; Endler and Mielke 2005; Stoddard and Prum 2008) use quantum cone catches for each of the cones in a bird’s retina to calculate a locus for the reflectance spectrum within the 3-dimensional colour space representing what a bird can see. Each locus calculated from a reflectance spectrum can be described by $r$, $\phi$ (hue latitude), and $\theta$ (hue longitude) within the avian tetrahedral colour space (Figure A1). These models make no assumptions about the light environment, the other optical properties of the birds eye, or the neural processing involved in the perception of colours, and thus calculate the potential signal that a bird can perceive.

FIGURE A1. Tetrahedral colour space based on a physiological model of avian vision. Each colour locus within this tetrahedron is determined by the relative stimulation of the uv/violet (U/V), long wave- (L), medium wave- (M) and short wave- (S) sensitive cones in the bird’s retina. Chroma is the length of the vector $r$, and achieved chroma is the length of $r$ relative to the maximum possible $r$ along that vector within this colour space. Hue is measured as both $\phi$ (the angle relative to the vertical vector) and $\theta$ (the angle relative to the horizontal vector). From Figure 3 in Endler and Mielke (2005).
A.3 Details of analyses

A preliminary analysis of the relations between colour variables measured from eyespots revealed two clear outliers (Figure A2) that had particularly low chroma values, and were often outliers in residual plots. We removed these two males from all analyses presented in the main text and this supplement. Because the eyespots of those two males had several extreme colour variable scores, including them in the analyses mainly strengthened the relations reported and gave more weight to the chroma values in statistical models.

FIGURE A2. Scatterplot of the relation between achieved chromas of the blue-green patch measured at illumination angles of 30° and 60° to the feather surface. Two clear outliers are circled in red. Data are plotted for 50 eyespots from 36 males (one eyespot per male per year).
Table A1 below summarizes the top models to predict male copulation success, based on different sets of variables calculated from the tetrahedral colour space model. The averaged model from each set is summarized in Table 2.1 in Chapter 2.

In the ‘all variables’ set, all of the top models included iridescence of the blue-green patch \((\phi \text{ at } 60^\circ \text{ vs } 30^\circ); \text{ see Chapter 2}\) as a predictor of male copulation success. Evidence ratios for the best model versus the other two top models range from 1.5 to 2.1 indicating that all 3 models are plausible and there is no compelling reason to choose among them, given the data. The averaged model (Table 2.1 in Chapter 2) echoes this conclusion with the iridescence and hue \((\theta)\) of the blue-green patch having the highest relative importance.

**Table A1.** Top ranked models \((\Delta AICc \leq 2)\) to predict peacock copulation success from iridescence, colour contrasts and both hue \((\phi \text{ and } \theta)\) and achieved chroma measured from three eyespot colour patches. Only the top 5 of the 20 top models for the bronze-gold patch are listed here, and all the rest had very low \((\leq 0.01)\) model weights.

<table>
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<th>coefficient</th>
<th>(\Delta AICc)</th>
<th>weight</th>
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<td>0.11</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>no. of eyespots</td>
<td>0.23</td>
<td>0.77</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>phi at 60°</td>
<td>1.86</td>
<td>0.79</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>no. of eyespots</td>
<td>0.22</td>
<td>1.40</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>phi at 60°</td>
<td>2.27</td>
<td>1.86</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bronze-gold</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>achieved chroma at 45°</td>
<td>0.26</td>
<td>0</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>achieved chroma at 45°</td>
<td>0.24</td>
<td>0.06</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>no. of eyespots</td>
<td>0.21</td>
<td>0.22</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

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Residual plots for all of the models presented in Table A2 indicated that residuals were very close to normally distributed (e.g., Figure A3).

<table>
<thead>
<tr>
<th></th>
<th>achieved chroma at 45°</th>
<th>achieved chroma at 60°</th>
<th>no. of eyespots</th>
<th>achieved chroma at 45°</th>
<th>theta at 60°</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.21</td>
<td>0.15</td>
<td>0.23</td>
<td>0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>no. of eyespots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>all variables</td>
<td>0.21</td>
<td>0.76</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

|   | no. of eyespots        |                        |                 |                        |             |
|   | blue-green phi at 60°  | 1.71                   | 1.51            | 0.16                   |             |
|   | blue-green phi at 30°  | –1.95                  | –2.02           | –2.57                  |             |
|   | blue-green theta at 60°| –2.49                  | –1.80           | –2.45                  |             |
|   | blue-green theta at 30°| 2.82                   | 1.64            | 2.68                   |             |
|   | bronze-gold achieved chroma at 45° | 0.13             |
TABLE A2. Morphology, behaviour and mating success (mean [95%CI]) for males in the eyespot colour manipulation experiment. The control group had naturally-coloured eyespots visible to females, whereas in the treatment groups the purple-black and blue-green patches were masked with black- or white-coloured stickers.

<table>
<thead>
<tr>
<th></th>
<th>control (n = 4)</th>
<th>black (n = 5)</th>
<th>white (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>train length (cm)</td>
<td>122.3 [109.5, 135.1]</td>
<td>121.6 [110.4, 132.8]</td>
<td>128.8 [119.5, 138.1]</td>
</tr>
<tr>
<td>number of eyespots</td>
<td>152.8 [147.7, 157.9]</td>
<td>149.6 [139.8, 159.4]</td>
<td>157.8 [153.0, 162.6]</td>
</tr>
<tr>
<td>displayed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>behaviour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>attendance (proportion of time)</td>
<td>0.79 [0.66, 0.92]</td>
<td>0.75 [0.61, 0.89]</td>
<td>0.76 [0.66, 0.86]</td>
</tr>
<tr>
<td>preen rate (proportion of time)</td>
<td>0.029 [0.010, 0.048]</td>
<td>0.039 [0.011, 0.067]</td>
<td>0.034 [0.005, 0.063]</td>
</tr>
<tr>
<td>display rate (proportion of time)</td>
<td>0.43 [0.37, 0.49]</td>
<td>0.27 [–0.01, 0.55]</td>
<td>0.28 [0.12, 0.44]</td>
</tr>
<tr>
<td>residual display rate</td>
<td>0.057 [–0.033, 0.147]</td>
<td>–0.046 [–0.096, 0.004]</td>
<td>0.0005 [–0.153, 0.154]</td>
</tr>
<tr>
<td>train-rattling bouts (/h)</td>
<td>8.63 [–3.15, 20.41]</td>
<td>3.91 [–0.12, 7.94]</td>
<td>1.79 [0.80, 2.78]</td>
</tr>
<tr>
<td>mating success</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female visitation rate</td>
<td>0.19 [0, 0.38]</td>
<td>0.10 [0.04, 0.16]</td>
<td>0.048 [0.020, 0.076]</td>
</tr>
<tr>
<td>copulation attempts (/h)</td>
<td>0.92 [–0.01, 1.95]</td>
<td>0.14 [0, 0.28]</td>
<td>0.075 [–0.069, 0.219]</td>
</tr>
<tr>
<td>copulations (/h)</td>
<td>0.34 [–0.04, 0.72]</td>
<td>0.019 [–0.034, 0.072]</td>
<td>0.0</td>
</tr>
</tbody>
</table>

A.4 References


APPENDIX B

SUPPLEMENTARY MATERIALS TO CHAPTER 3

The figures on the following two pages in this Appendix illustrate encounter sequences for 8 different females that were observed copulating, 4 in 2009 and 4 in 2010.

These encounter sequences are illustrated as timelines (one per female) showing all skips, visits, and copulation events observed for each female over the span of time that observations were conducted. Skips are defined as males encountered with their trains erect that were not visited. Encounters where the males did not have an erect train are not shown on these figures. The size of the circles for visit events corresponds to the total amount of time the female viewed the male’s train-rattling display (see legend), and colours indicates male identity (using a consistent scheme across both figures). Events are spaced evenly within each day along the horizontal axis for clarity.
APPENDIX C

REPEATABILITY OF COLOUR MEASUREMENTS

To determine the repeatability of our measurements of eyespot colours, we examined spectral data from 82 different *P. cristatus* eyespot feathers collected from 54 adult males (taking the single most symmetrical feather collected per male in each year that he was captured). Since we were interested here in the agreement repeatability of our methods on a feather-by-feather basis in order to evaluate measurement error, we considered each feather to be an independent sample, even though some were from the same male captured in the Los Angeles Arboretum population in more than one year.

Table C1 below gives the intraclass correlation coefficient (ICC) values for the tristimulus colour variables (brightness, chroma and hue) for each colour patch at each illumination angle (30°, 45° and 60°), estimated from the variance components of a one-way ANOVA using the R package ICC. Tristimulus colour variables were calculated using the methods described in Chapter 2.

As shown in Table C1, estimates of brightness and chroma were highly repeatable for all three colour patches at all three measurement angles (all ICC > 0.81, $\alpha = 0.05$). Hue, or the wavelength of peak reflectance, was also highly repeatable for the bronze and blue-green patches (all ICC > 0.86, $\alpha = 0.05$), but somewhat less so for the dark purplish-black patch (all ICC > 0.61, $\alpha = 0.05$). This is not surprising, given that the relatively weak reflectance from this patch may have been exceeded by spectral noise in the UV region occasionally (e.g., see Figure 2.2 in Chapter 2 or Figure 4.3 in Chapter 4). Overall,
the high values for ICCs in Table C1 indicate that our methods for taking spectral measurements yielded very consistent estimates of feather colour.

**TABLE C1.** Repeatability of tristimulus colour variables (brightness, chroma and hue) calculated from two independent spectral measures of the same spot on each eyespot feather (n = 82 feathers). The table gives intraclass correlation coefficients (ICCs) followed by the lower bound of the 95% confidence interval for this statistic. The sample of 82 feathers includes the single most symmetrical eyespot feather removed each adult male captured in 4 years of study, and it includes pseudoreplication of males in the LAA population that were captured in >1 years.

<table>
<thead>
<tr>
<th></th>
<th>Brightness ICC, lower bound</th>
<th>Chroma ICC, lower bound</th>
<th>Hue ICC, lower bound</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bronze</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60°</td>
<td>0.95, 0.92</td>
<td>0.87, 0.81</td>
<td>0.94, 0.91</td>
</tr>
<tr>
<td>45°</td>
<td>0.97, 0.96</td>
<td>0.93, 0.89</td>
<td>0.96, 0.93</td>
</tr>
<tr>
<td>30°</td>
<td>0.93, 0.89</td>
<td>0.90, 0.85</td>
<td>0.91, 0.86</td>
</tr>
<tr>
<td><strong>Blue-green</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60°</td>
<td>0.99, 0.99</td>
<td>0.97, 0.96</td>
<td>0.91, 0.87</td>
</tr>
<tr>
<td>45°</td>
<td>0.99, 0.98</td>
<td>0.99, 0.99</td>
<td>0.97, 0.95</td>
</tr>
<tr>
<td>30°</td>
<td>0.97, 0.95</td>
<td>0.98, 0.97</td>
<td>0.97, 0.96</td>
</tr>
<tr>
<td><strong>Purple-black</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60°</td>
<td>0.99, 0.98</td>
<td>0.94, 0.91</td>
<td>0.73, 0.61</td>
</tr>
<tr>
<td>45°</td>
<td>0.99, 0.98</td>
<td>0.97, 0.95</td>
<td>0.78, 0.67</td>
</tr>
<tr>
<td>30°</td>
<td>0.99, 0.98</td>
<td>0.94, 0.91</td>
<td>0.73, 0.62</td>
</tr>
</tbody>
</table>
APPENDIX D

MODELING PERCEPTION OF SIMULATED EYESPOT COLOURS

D.1 Methods

To understand how spectral properties affect the colour contrast of peacock eyespots, and to investigate the perceptual effects of a wide variety of eyespot colours under various light conditions, we simulated a range of eyespot colour spectra that were similar to the ones we measured from actual peacock feathers. These simulated spectra are illustrated in Figure D1 below.

To produce these simulated spectra, we began by using the normal distribution probability function in R to generate unimodal curves ranging from 300-700 nm, and then we transformed these curves to have approximately the same shape as the spectra measured from actual peacock feathers. For the bronze eyespot colour, two unimodal curves were combined to generate a bimodal curve with approximately the same shape as the bronze colour spectrum, smoothing the area where the two curves were joined with a spline smoothing function.

We first generated curves with the approximate brightness and chroma (i.e., maximum reflectance and spectral purity or “peakiness”) of the average *P. cristatus* eyespot spectra at a 45° illumination angle (which are shown in Figure D1a). We used a minimum reflectance of 5% for these spectra. We then generated a series of hue shifts by shifting these curves to the left and right by 2 nm increments. In total, we generated 27 hue-shifted spectra for each eyespot colour patch (purple-black, blue-green and bronze) such
that each set of simulated colour spectra encompassed the average hues of \emph{P. cristatus} and \emph{P. muticus}, as well as more extreme hues beyond those seen in actual peacock eyespots (Figure D1c). We refer to this as the base set of simulated spectra.

Next, we manipulated brightness by multiplying every spectrum in the base set by a constant. In this way, we generated two sets of spectra with enhanced brightness relative to our base set: one intermediate brightness set, using a constant multiplier of 1.5 to achieve brightness in between that of \emph{P. cristatus} and \emph{P. muticus} (Figure D1d), and one maximum brightness set, using a constant multiplier of 2 to achieve the approximate brightness of \emph{P. muticus} colours (Figure D1e). These brightness-manipulated spectra have slightly higher minimum reflectance values than our base set (7.5% and 10%, relative to 5% for the base set).

We then manipulated chroma of the base set using a logarithmic function, by adding the expression \(k\times\log(y-4)\) to each reflectance value \(y\). This allowed us to increase the height and steepness of the curves while keeping the minimum reflectance constant at 5%. We chose values of \(k\) separately for each colour patch to achieve two chroma-enhanced sets: one intermediate chroma set with spectral shape in between that of \emph{P. cristatus} and \emph{P. muticus} (Figure D1f), and one maximum chroma set with spectral shape similar to that of \emph{P. muticus} (Figure D1g). It should be noted that both types of manipulations we used would affect spectral brightness as well as chroma, but that the main difference between them was that the chroma manipulations altered spectral intensity without raising the level of minimum reflectance.
FIGURE D1. Real (a-b) and simulated (c-g) eyespot colour spectra. (a-b) Average spectra for the three main colour patches from (a) *P. cristatus* and (b) *P. muticus* eyespot feathers, measured at a 45° light angle. (c) Simulated spectra with the approximate brightness and chroma of *P. cristatus*, and 2 nm hue shifts, showing 27 hue-shifted colour spectra for each colour patch.
FIGURE D1 cont. (d-g) Simulated spectra with (d-e) enhanced brightness and (f-g) enhanced chroma, and 2 nm hue shifts. Each panel shows 27 hue-shifted colour spectra for each colour patch.
Using these simulated spectra, we calculated pairwise perceived colour contrasts under mid-morning/mid-afternoon D55 light conditions expected to be typical of *P. cristatus* displays in open habitats, following the methods described in Chapter 4.

**D.2 Effect of changes in brightness**

Using our brightness-manipulated spectra, and considering a range of possible hues for each brightness level (base, intermediate, maximum; Figure D1c,d,e) under open habitat light conditions, we found no effect of brightness level on any of the three eyespot colour contrasts (i.e., bronze vs. blue-green, blue-green vs. purple-black, and purple-black vs. bronze; all $F_{2, 6558} < 0.0001$, all $p > 0.999$, $n = 6561$ for each colour contrast). Thus, increasing the brightness of the eyespots across all wavelengths does little to enhance perceived chromatic contrast; or in other words, spectral purity is critical for high chromatic contrast between these particular colours.

**D.3 Effect of changes in chroma**

Using our chroma-manipulated spectra, and comparing each chroma level (base, intermediate, maximum; Figure D1c,f,g) while considering a range of possible hues, we found that increasing chroma generally increased the eyespot colour contrasts (Table D1). However, there were some exceptions.
**TABLE D1.** Effect of chroma manipulations on eyespot colour contrasts (df = 2, 6558).

<table>
<thead>
<tr>
<th>Colour patch</th>
<th>Effect on chromatic contrast</th>
<th>direction</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronze</td>
<td>vs. blue-green</td>
<td>+</td>
<td>8.8</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>vs. purple-black</td>
<td>+</td>
<td>243</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Blue-green</td>
<td>vs. purple-black</td>
<td>+</td>
<td>1660</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>vs. bronze</td>
<td>+</td>
<td>646</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Purple-black</td>
<td>vs. bronze</td>
<td>+</td>
<td>1.2*10^4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>vs. blue-green</td>
<td>–</td>
<td>625</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Specifically, for the bronze patch, increasing its chroma also increased its contrast with purple-black patch, but it did not substantially affect its contrast with the blue-green patch. Although the positive effect in Table D1 is significant, it is extremely small and is significant due to the very high sample size of 6561 contrasts.

For the purple-black patch, increasing its chroma increased its contrast with the bronze patch, while decreasing its contrast with the blue-green patch. In other words, the greatest blue-green vs. purple-black contrast is achieved when purple-black chroma is minimized and blue-green chroma is maximized. Increasing the chroma of the blue-green patch increased its contrast with each of the other two patches.

**D.4 Effect of hue shifts**

We used the base set of spectra shown in Figure D1c to examine the effect of hue shifts on each of the eyespot colour contrasts. As shown in Figure D2a, maximum bronze vs.
blue-green contrast is achieved when the blue-green patch is shifted towards shorter wavelengths (shorter than that of both *P. cristatus* and *P. muticus*). At the same time, shifts in the bronze hue had little effect on this contrast (Figure D2a). The blue-green vs. purple-black contrast is maximized when the blue-green hue is shifted towards longer wavelengths and the purple-black hue is shifted towards shorter wavelengths (Figure D2b), as seen in *P. muticus*, although it should be noted that maximum contrast is achieved at more extreme hues than those seen in actual *P. muticus* feathers. Lastly, the purple-black vs. bronze contrast is maximized when the purple-black hue is shifted towards longer wavelengths (longer than that of both species) and the bronze hue is shifted towards shorter wavelengths (shorter than that of both species; Figure D2c).

Thus, for both the blue-green and purple-black patches at least, there is a no single hue that would maximize both of its pairwise contrasts with the other two eyespot colours in open habitat light conditions. In other words, shifting the hue of these colours in either direction will have opposing effects on its two pairwise contrasts. For the bronze patch, however, shifting its hue towards shorter wavelengths would increase its contrast with both of the other two eyespot colours, although interestingly, changing the bronze patch hue has very little effect on its contrast with blue-green under open habitat light conditions (Figure D2a).

**D.4 Comparison with forest shade conditions**

We repeated all of the analyses described above using forest shade conditions expected to be typical of *P. muticus* habitat (see Chapter 4 for details). We found the same results for
brightness and chroma manipulations described above, but results for hue shifts were slightly different (Figure D3), primarily in terms of the effects on the bronze vs. blue-green contrast (e.g., compare Figure D3a to D2a).

Under forest shade conditions, unlike open habitat light, shifting the blue-green hue to shorter wavelengths has little effect on the bronze vs. blue-green contrast. Moreover, maximal contrast can be achieved with a blue-green hue equivalent to that of actual *P. muticus* feathers under forest shade. Additionally, the bronze hue appears to have a stronger affect on this contrast in forest shade conditions, compared to open habitat light.
FIGURE D2. Colour contrasts for simulated eyespot colours under D55 light conditions.
FIGURE D3. Contrasts for simulated eyespot colours under forest shade conditions.
**D.6 Summary**

These simulations demonstrate that for these nanostructural colour spectra:

i. Increasing spectral reflectance (or brightness) over all wavelengths has little effect on perceived colour contrasts;

ii. Increasing peak intensity while maintaining the same minimum reflectance (i.e., increasing chroma) generally increases perceived colour contrasts, with some exceptions:
   a. Increasing bronze chroma has little effect on its contrast with blue-green
   b. Increasing purple-black chroma decreases its contrast with blue-green

iii. In terms of hue shifts:
   a. Changes in the purple-black and blue-green patches may have opposing effects on the two pairwise colour contrasts involving these patches.
   b. Under open habitat light conditions, it is possible to achieve near-maximum blue-green vs. bronze contrast with hues equivalent to those of actual *P. cristatus* eyespots. Interestingly, shifts in the bronze hue in these open habitat light conditions have little to no effect on this contrast.
   c. Under forest shade conditions, it is possible to achieve maximal blue-green vs. bronze contrast with a blue-green hue equivalent to that of *P. muticus*. Thus, *P. muticus* is able to achieve greater blue-green vs. purple-black contrast while maintaining high blue-green vs. bronze contrast in its darker forested environment.
Overall, these results reinforce the idea that *P. muticus* and *P. cristatus* eyespots are tuned to maximize colour contrast under different light conditions, in a manner that may strike a balance between different colour contrasts in the pattern. Furthermore, the results of these simulations suggest that, for these nanostructural colours at least, any changes that enhance chroma, or spectral purity, should be much more effective at increasing perceived chromatic contrast than any changes that affect brightness or the overall amount of light reflected.