Investigating patterns of avian ornamental colouration: Intraspecific and interspecific approaches

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Figure 1-1 Male blackbird (Turdus merula) photographed on Massey University Campus, Auckland, showing colour bands used for individual identification of males used in chapter two. Photo: Millie Ahlstrom.

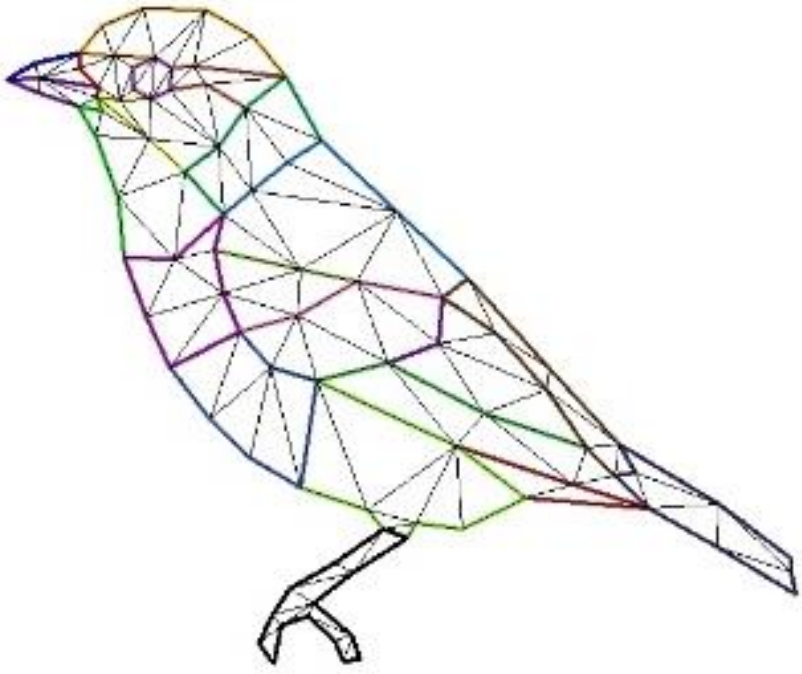

Figure 1-2 Whole bird reference template created by a novel method in chapter three. This template removes the morphological variation between birds, allowing direct comparisons of colouration to be made between homologous regions of the body. Image: Millie Ahlstrom

ABSTRACT

Research into the evolutionary function of elaborated colouration in birds is continually ongoing. Novel approaches to addressing various aspects of this broad research area may help us consider this topic in a new light. This thesis aimed to consider two different aspects of avian colouration research using novel methods and testing relatively new hypotheses. Firstly, I consider the cost of carotenoid pigmented ornamental colouration - an area of research that is currently under intense debate. Classically carotenoid-based pigmentation has considered carotenoids to function as indicators of sexual quality, with costs being due to carotenoids being diet dependent in birds. Recent research however has argued that carotenoid pigmented colour traits function in agonistic social contexts, and that the cost of using carotenoid pigmentation as an honest indicator of quality is a social one. In this study I test this hypothesis using blackbirds (*Turdus merula*) and their natural variation in carotenoid-based bill colouration. I replicate a study using model presentations to simulate territory intrusions. Additionally, I examine the feasibility of using three-dimensionally printed models in avian behavioural studies. This study was unsuccessful due to a lack of response rates from territorial males, however it was successful in questioning several differing aspects between my study and the study I replicated. Secondly, I explore the spatial organisation of colouration on the bodies of birds as a way of potentially inferring different functions of elaborate colour traits. In the third chapter I develop a novel method that allows objective analysis of the spatial organisation of colour on the bodies of birds, by removing morphological variation between species. Using this method I present a case study on the spatial organisation of colour elaboration in 2,471 species of passerines. This case study uses a difference in sexual dichromatism as a proxy for colour elaboration and determined where signaling hotspots occur on the bodies of birds. These results demonstrate that conspicuous colouration is most common in the supercilium, chin, and upper breast of passerine birds. In chapter four, I used this method to determine correlations between different life-history traits and different regions of the body. This study aimed to infer the function of different regions of the head in signaling. My results show that the irises in species with tropical life-histories and cooperative breeding strategies are more likely to be elaborately coloured; bills of larger bodied species are more likely to be elaborately coloured than are smaller species; and the spatial organisation of colour effects females more than it does males.

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Chapter 1 A general introduction and literature review of colouration in birds

1.1 Abstract

Bird colouration has evolved to perform several different communicative functions. These functions range from camouflage, obtaining a mate, to winning competitive contests and much more. The diversity of avian colour is produced by different mechanisms in birds, either by different pigments or different structural arrangements, and can occur both in bare parts of birds as well as in plumage. Quantification of colouration in birds has been performed in a variety of different ways. This different interpretation of colour creates differing opinions of what is "bright" colouration and what is a dull colouration. These opinions create confusion within bird colouration research due to results gained from different methods not being comparable. Avian colouration research is therefore a multifaceted research topic when considering the different functions, mechanisms, and measurements of colour. This review attempts to assemble these different aspects of avian colouration in a logical manner whilst focusing predominantly on the use of colour traits in social signalling, ways to quantify colour, and the spatial organisation of colour in birds.

1.2 Introduction

Functions of communication in birds include species recognition (Delhey, Peters, & Kempenaers, 2007); attracting mates; competing for resources; group cohesion; and minimising predation risk (J. A. Endler, 1992). Communication is defined by the exchange of information between two or more participants. This information can be exchanged through several different channels including auditory, visual (colour as well as displays) (Marler, 1957), olfactory (Balthazart, Taziaux, Keller, & Bakker, 2009) and tactile communication (Avilova, Fedorenko, & Lebedeva, 2018). The type of communication used depends largely upon the environment that the animal occurs in. The environment acts as a vector to carry a signal and can thus alter or degrade a signal (G. G. Rosenthal & Ryan, 2000). The environment therefore poses a huge selection pressure on the form of communication a population or species uses (Rundus & Hart, 2002; Slabbekoorn & Smith, 2002).

The social environment of a species also poses a selection pressure on communication type. The anatomy of a signal recipient for example will hugely influence signal type. For example, New Zealand native frogs do not have tympanum (ear drums) and are non-vocal in their communication (Waldman, 2000) as vocal communication would not be successful in communicating information. There are therefore constraints to which type of communication can and will evolve in different species. Due to light being prevalent in most environments, light receptor organs were quick to evolve in many different phyla (Osorio & Vorobyev, 2008). These organs have evolved different capacities and exist in different shapes and form (Lamb, 2013), but are none the less common organs. The use of light and vision in day-to-day activities associated with survival are what made light receptor organs a vital part of anatomy (Osorio & Vorobyev, 2008). This prevalence of visual capabilities in conspecific and heterospecific species was an important prerequisite for the evolution of using light in a communication context. Visual communication is widespread through different taxa, this is reflected by the amount of colour present in many plant, invertebrate, and vertebrate species (Osorio & Vorobyev, 2008). Visual signals in the form of colour radiate relatively far (J. A. Endler, 1992) and can therefore reach receivers from a considerable distance (as long as there is light present).

Birds are one of the most colourful taxa of animals as they use colour displays in visual communication. Birds are therefore often studied in regards to their use and production of colour (Shawkey, Morehouse, & Vukusic, 2009). Colour is the absorption and reflection of different wavelengths of light, where those that are reflected are perceived as colour (J. A. Endler, 1990). This reflection of light (and thus production of colour) is produced by three main mechanisms in birds: structural mechanisms, pigments or a combination of both (Riedler, Pesme, Druzik, Gleeson, & Pearlstein, 2014). Structural colours are produced by different physical structures in feathers which reflect light, producing blue and iridescent colours (Riedler et al., 2014). When these structures act alone, all light is reflected creating white colour. However, these structures often act in concert with pigments to create several different colours (Riedler et al., 2014). There are four main types of pigments in birds: carotenoids, melanins, psittacofulvins, and porphyrins (Krishnaswamy & Sundaresan, 2012). Each of these pigments produce a variety of different colours and do so by differentially absorbing and reflecting different wavelengths of light (Prum, Andersson, & Torres, 2003).

The most common colour pigments in birds are carotenoid and melanin pigments (Krishnaswamy & Sundaresan, 2012). Carotenoids produce yellow, orange, and red colouration (García-de Blas, Mateo, & Alonso-Alvarez, 2016) whereas melanin pigments create colours that range from yellow-brown to black (Eliason, Shawkey, & Clarke, 2016). Melanin pigments are considered to be the most ancestral colour pigment of birds (Stoddard & Prum, 2011) and are synthesised from amino acids in feathers (Eliason et al., 2016). Carotenoids on the other hand are the only pigment that cannot be synthesised by birds and are acquired through the diet (García-de Blas et al., 2016). Both carotenoid and melanin pigmentation are common throughout the avian phylogeny. The other colour pigments (psittacofulvins and porphyrins) are less common as they are family specific. Psittacofulvins occur only within the parrot (Psittacine) family where they produce yellow, orange, and red colouration. Unlike carotenoids however they are not diet dependent (Berg & Bennett, 2010) and instead are believed to be synthesised within the feathers of parrots (McGraw & Nogare, 2004). Porphyrin pigments, which produce pink and brown colour, are also family specific, occurring only in the Strigiformes, Caprimulgiformes, and Gruiformes (Galvan, Camarero, Mateo, & Negro, 2016). This pigment is synthesised in the bodies of these birds, and (unlike melanin and psittacofulvin) is present in the blood (as opposed to being restricted to the feathers) (Galvan et al., 2016). A modified version of this pigment called metalloporphyrin (which contains iron or copper metals (Riedler et al., 2014)) produces green and purple hues and is restricted to the Musophagidae family (Krishnaswamy & Sundaresan, 2012).

The various pigments outlined above do not occur independently of one another and can therefore be present simultaneously within a species and an individual. Different types of pigmentation within one species can act to create within-species variation either by incorporating different colour patches within the plumage of one bird, or through sexual dichromatism and polymorphism. Sexually dichromatic species are those in which the male and female of the species are coloured differently (Badyaev & Hill, 2003b); such as in mallard ducks (*Anas platyrhynchos*), blackbirds (*Turdus merula*), and ring-necked pheasants (*Phasianus colchicus*). Polymorphic species are those where two or more differently coloured (and thus genetically different) morphs can occur within a population or a species (Galeotti, Rubolini, Dunn, & Fasola, 2003); such as the Australasian magpie (*Cracticus tibicen*) and the New Zealand fantail (*Rhipidura fuliginosa*). This within-species variation of

colour dramatically increases the variation that exists within the avian taxa.

The extreme variation in bird colouration has evolved through several mechanisms: natural selection (predation avoidance), sexual selection (mate choice and mate competition), and social selection (intraspecific competition) (Baker & Parker, 1979; West-Eberhard, 1983). Sexual selection is the most common hypothesis for explaining colourful and elaborate ornaments in animals. Darwin (1871) hypothesised that female preference for elaborate ornaments is what drives selection for these traits in males. Elaborate traits are found in both sexes however. This can be explained by the two partitions of sexual selection: intrasexual selection and intersexual selection. Intrasexual selection describes the competition that occurs between members of the same sex for resources (which includes mates, hence the sexual selection aspect (Chaine, Tjernell, Shizuka, & Lyon, 2011)). This competition selects for elaborate plumage traits in both males and females. Traits that are not explained by some aspect of sexual selection are most likely explained by natural selection. Natural selection is the selection of certain traits that increase the survival and reproductive rates of a species (O'Donald, 1972). This can select for dull and inconspicuous plumage used as camouflage, or for bright and conspicuous plumage as a sign of unpalatability (Baker & Parker, 1979). When we consider these selection pressures separately, they are logical. When considering these pressures together, it becomes unclear how conspicuous colouration evolves when natural selection selects for cryptic colouration.

Delhey (2019) recently demonstrated that there is a trade-off between cryptic colouration and conspicuous colouration. He found that conspicuous colours were constrained to certain parts of the birds' bodies, where he suggested that the colours would be less visible to unintended receivers (such as predators). This demonstrates that different evolutionary pressures can act in concert and that this results in different colour patches within an individual bird. Different colour traits in birds have previously been considered in isolation. More recently, it is becoming clear that different colour traits within one individual can function to convey different types of information (Chaine & Lyon, 2008). This is an area of research that is growing and aims to understand how the different evolutionary pressures act together to create the huge colour diversity of birds.

1.3 Methods of colour quantification in birds

Birds have different visual capabilities than humans do, so it is important to take this into consideration when interpreting both the colouration of birds and the potential function of those colours. Birds have tetrachromatic vision, meaning that they have three similar colour cones to humans, but in addition, they have a cone that allows them to see in the ultraviolet (UV) spectrum (Bowmaker, Heath, Wilkie, & Hunt, 1997). Additionally, the presence of oil droplets on the cone cells of birds makes distinguishing between changes in colour shades more efficient for birds than for humans (Bowmaker et al., 1997). This means that the range of colours visually available to birds are different to those that are available to us. Several studies have consequently examined whether human-mediated vision can accurately quantify avian colouration (Armenta, Dunn, & Whittingham, 2008; Bergeron & Fuller, 2018; Seddon, Tobias, Eaton, Ödeen, & Byers, 2010). One particular study revealed that even when considering UV variation in plumage, human perception of whether a species was sexually dichromatic or not was comparable to quantitative data (Armenta et al., 2008, this study included 900 species of birds). Human vision therefore recognises the majority of colour variation in birds (Bergeron & Fuller, 2018). One issue with this however is when we

consider large scale studies: human eyes tire easily which can introduce error in the data (Ukai & Howarth, 2008).

Studying colouration in birds on a large scale is therefore more accurate and efficient when using methods other than human perception. The physical properties of light make it a variable that is easily measured and quantified (J. A. Endler, 1990). A spectrophotometer is a piece of equipment that allows us to quantify colouration by measuring the strengths of a wavelength of light – i.e. colour. This equipment can measure light in the UV spectrum and so considers all avian vision capabilities. This is a method that has been used widely in avian colouration studies because of its accuracy (Bergeron & Fuller, 2018; Cuervo & Belliure, 2013; McNett & Marchetti, 2005). There are drawbacks to using spectrophotometry however, hence other methods of colour quantification are often adopted. Spectrophotometry requires physical access to the bird to be able to measure colour. For interspecific work, museum specimens are typically used. However, taxidermy models have been shown to fade in colour over time (McNett & Marchetti, 2005) which potentially excludes the use of these specimens in some studies. Acquiring measurements from live specimens is therefore often necessary. Due to measurements in the field reflecting the most accurate colour of plumage (Vaquero-Alba, McGowan, Pincheira-Donoso, Evans, & Dall, 2016), these studies need to be carried out in the natural habitat of the birds. This is sometimes unachievable, especially for studies of large numbers of species. Spectrophotometry is also limited when it comes to studying colouration in bare parts, as flattened areas are required for successful measurement (such as flattened feathers) (Villafuerte & Negro, 1998).

The complications of colour quantification using spectrophotometry can be overcome by using other methods. Digital photographs (Villafuerte & Negro, 1998) and illustrations (Galvan & Moller, 2011; Miller, Leighton, Freeman, Lees, & Ligon, 2018; Stang & McRae, 2009) of species scanned into computers can be used to measure colouration. This method solves issues associated with access to specific species (this considers physical access due to environment as well as access restrictions associated with research consents). Digital images in computer space are displayed using computer visual systems which represents colour using hue, saturation, and brightness (HSB) values, or red, green, and blue (RGB) values. HSB values indicate the tint (hue), the saturation of that colour (for example red is more saturated than pink), and the total reflectance of that colour or surface (brightness), which ultimately indicates whether the colour is white, black or in-between those two extremes (McNett & Marchetti, 2005). Colour represented by RGB values gives three values on a scale of 0 to 255, where the amount of each channel present in a colour is indicated (Abdolmaleky, Naseri, Batle, Farouk, & Gong, 2017; Hill, 1998). For example, the colour red is denoted as 255, 0, 0, which means that there is the maximum amount of red colour, no green colour, and no blue colour present. The method of measuring colour in this way removes the UV component of that plumage colour, however this is nevertheless a method that has been validated (Dale, Dey, Delhey, Kempenaers, & Valcu, 2015; Miller et al., 2018).

HSB and RGB values are quantitative measures of colour, however the interpretation of what a given value represents is often qualitative. For example, the colours black and white can be considered to be dull in comparison to other colours, but in situ on a bird (the magpie (*Cracticus tibicen*) for example), these colours are startling and contrasting to their environment. Depending on which study you read, black and white will either be classed as dull (Dunn, Armenta, & Whittingham, 2015) or bright (Mason, Shultz, & Burns, 2014).

Sometimes “brightness” (black or white) isn’t considered at all (Stoddard & Prum, 2011). What is considered as a colourful bird can be up for interpretation. Birds like the Gouldian finch (*Erythrura gouldiae*) are, in my opinion, a colourful bird due to the seven different colours arranged all over their bodies. Some studies will average the colour across different regions of the body however, which would result in birds like the Gouldian finch being represented as dull in colour (Dunn et al., 2015). Other studies do however consider plumage complexity when interpreting colouration (Mason et al., 2014). These examples demonstrate the huge diversity in interpretation of what colour means within birds, which make these studies, and therefore their results, incomparable.

More recently, tetrahedral colour space is a method that has been used in several studies (J. A. Endler & Mielke, 2005; Mason et al., 2014; Stoddard & Prum, 2011). The common use of this method allows for comparisons between studies as well as an overall agreement of the qualities of different colours. Tetrahedral colour space is a three dimensional colour space which incorporates all colours that occur within the tetrachromatic vision of birds (Stoddard & Prum, 2008). All plumage colours are therefore considered when using this method, and there is a consistency in the quantitative measurements as a result. Tetrahedral colour space processes chromatic and achromatic colours (these are colours with and without hue and saturation, respectively) separately (Stoddard & Prum, 2011) due to achromatic and chromatic vision being processed separately in the brains of birds (Kelber, Vorobyev, & Osorio, 2003; Osorio & Vorobyev, 2005). Achromatic vision is predominantly used for detecting movement, where colour vision is used for behaviours such as photo-taxis and object recognition (Kelber et al., 2003). Achromatic colours are often used in conspecific signalling however, to signal information such as dominance (Rohwer & Rohwer, 1978; Woodcock, Rathburn, & Ratcliffe, 2005), sexual quality (Woodcock et al., 2005) and predator alarm (Stang & McRae, 2009). This demonstrates that when studying colour elaboration in birds, achromatic and chromatic colours should be considered together and therefore even this method has deficits. The presence of various methods and interpretations of colour quantification need to be resolved to better understand the function of colouration in birds.

1.4 Social signalling in birds – badges of status

Males and females of the same species will compete for resources such as food, territories, and mates (Chaine & Lyon, 2008). These competitions need to be won in order to gain access to the resource (West-Eberhard, 1983). Aggressive interactions can be a result of these competitions. An evolutionary response to this aggression are armaments (weapons), which are socially selected to be used in direct combat during competitions (Berglund, Bisazza, & Pilastro, 1996). Direct combat is a costly solution to competition however due to potential injury (West-Eberhard, 1983). Ornamental traits have evolved as a solution – ornaments are traits that signal the competitive ability of an individual without the costly escalation to aggression (Dey, Dale, & Quinn, 2014; Pryke & Andersson, 2003). When the cost of fighting is high, relative to the cost of the resource, the selection for these signals is higher (Johnstone & Norris, 1993). Socially selected ornaments in birds are often expressed as plumage traits, or bare part traits (such as bill colouration) and are referred to as ‘badges of status’.

Badges of status are often correlated with different indexes of vigour. For example, the extent of a badge is correlated with parasite load (Biard, Saulnier, Gaillard, & Moreau, 2010), hunting skills, and territory quality (Casagrande, Csermely, Pini, Bertacche, &

Tagliavini, 2006) in some species. Some traits can change colour within hours, which indicates that these badges are responsive to dynamic changes of condition (M. F. Rosenthal, Murphy, Darling, & Tarvin, 2012). A change in social environment (like social rank) can change the trait (Karubian, Lindsay, Schwabl, & Webster, 2011) and the behaviour of the individual (Iverson & Karubian, 2017). These in turn affect how an individual is perceived by competitors and consequently affects the level of aggression that is elicited towards that individual (Tibbetts, 2014).

For badges of status to be reliable and honest they need to carry a cost (Zahavi & Zahavi, 1999). This cost demonstrates that the individual can maintain their badge due to being a high quality individual, rather than due to chance (Dey et al., 2014). If there was no cost associated with these traits, any individual could acquire the trait, and it would thereby be a false representation of their quality. A great example of a trait that carries many putative costs is carotenoid-pigmented colouration. Originally, the cost of using carotenoids as a pigment was thought to be due to having to acquire this pigment through the diet (Griggio, Pilastro, Serra, Licheri, & Monti, 2007; Olson & Owens, 1998; Trigo & Mota, 2015). This meant that individuals that use carotenoids in a badge of status would have to be successful foragers to have bright colouration and therefore must be of high quality. It was subsequently discovered that carotenoids also function as immunostimulants and antioxidants (Griggio et al., 2007; Moller et al., 2000). There is therefore argued to be a trade-off between using carotenoids for pigmentation rather than for physiological function. If an individual is able to carry a brilliantly coloured badge of status it reflects their health as they do not require those carotenoids for physiological function.

Recent studies dispute these argued costs however and suggest instead that the honesty of a badge of status is enforced by social conflict (Chaine & Lyon, 2008). Carotenoid-pigmented badges of status are often used to resolve competitive interactions (E. S. A. Santos, D Scheck, & S Nakagawa, 2011a). Carotenoid-pigmented bills are used as status signals (Bolund, Schielzeth, & Forstmeier, 2007; Shawcross & Slater, 1984) which demonstrates that they are potentially a socially selected trait. The cost of using carotenoids for these traits may therefore be social rather than developmental (Griggio et al., 2007; Pryke, Lawes, & Andersson, 2001). Social costs arise through two scenarios: agonistic associations between individuals of similar signals and aggressive testing of individuals with mismatched signals (Tibbetts, 2014). Individuals that signal a badge that represents false quality (as determined by a mismatch of behaviour) are tested by others, which is where costs occur if an individual cannot uphold the aggression that is associated with the false badge (Tibbetts, 2014).

Physiological changes result as a consequence of social interactions (Tibbetts, 2014); this can result in changes in condition-dependent traits. Bare part signals (bills, wattles, legs etc) are more reliable than plumage traits in reflecting these changes. Plumage is constrained to changing colour during seasonal moults (Dey, Quinn, King, Hiscox, & Dale, 2017), which only happens either biannually or annually (depending on the species). Feather colour conditions can thus be an inaccurate indicator of an individual's health as feather colour reflects the condition of the individual during the growth of those feathers, not the current condition (Dey et al., 2014). However, because bare part signals can change colour within a few hours (M. F. Rosenthal et al., 2012), they reflect dynamic signals where colouration changes depending on the individual's current health (Ardia, Broughton, & Gleicher, 2010). Vascularised soft parts (bare parts) are therefore efficient signals that reflect current condition of an individual, due to changes in dominance, group composition or ecological

conditions (Karubian et al., 2011).

1.5 Using the spatial organisation of colour to infer function

The location of a signal on the body can hugely affect how the receiver of that signal views and therefore interprets the signal. The placement of a signal on the body considers interactions between conspecific and heterospecific species in how the body is displayed to the different signal receivers. For example, the female *Acanthodactylus erythrurus* lizard has conspicuous red colouration on the hind legs and tail (Cuervo & Belliure, 2013). This is an area of the body that is presented to the male when he is approaching to mate. This red colouration has been shown to fade to a white colour after the female becomes gravid, therefore serving as a courtship rejection signal. The location of this colour helps to signal the message as it is an area that is visible to the male when and if he tries to mate with the female. Another example is demonstrated by the chameleon, whose heads change colour during competitive interactions to reflect their competitive ability (Ligon & McGraw, 2013). During competitive interactions the head of an individual is constantly facing towards the rival, the signalling of competitive ability on the head ensures that this signal is visible to the rival individual during this interaction. These two examples indicate the importance of the location of a signal for the signal to be seen by the intended receiver. They also suggest that insight into the function of a certain signal can be gained through consideration of where the signal is located.

To expand on this idea, when considering the function of a certain colour trait it is also important to consider whether a certain area of the body may use movement to communicate certain information. For example, the male peacock spider (*Maratus volans*) performs a dance, used as a mating ritual (Girard, Kasumovic, & Elias, 2011). This ritual is sexually selected upon by females, as females prefer males that display for longer periods of time (Girard, Elias, & Kasumovic, 2015). The legs that are used in the mating dance display conspicuous colouration (this contrasts with colouration of the other legs) which is also a sexually selected trait (Girard et al., 2011). The mating displays of these spiders are therefore multi-modal where contrasting colouration in the displaying legs can help to accentuate the movement and therefore the signal (which is male quality). This increase the chance of information being transferred whilst reducing costs associated with signal failure and signal production created by communication failure (Cooney et al., 2019).

Further examples of the association between colour and movement are demonstrated in birds. Ultraviolet-reflective plumage was shown to be correlated with physical areas that are moved during courtship displays in a comparative study of 108 bird species (Hausmann, Arnold, Marshall, & Owens, 2003). This movement of parts of the body during certain displays is also commonly associated with bright or contrasting colouration. Wing presentations are often used as a part of mating displays in birds and colouration under the wings is often used to enhance the brilliance of these displays. This is demonstrated by the startling white colour under the wings of blue-black grassquits (*Volatinia jacarina*) (Maia, Brasileiro, Lacava, & Macedo, 2012) and the bright red colour under the wings of northern cardinal (*Cardinalis cardinalis*) females (Jawor & Breitwisch, 2003). These elaborate ornaments have both been revealed to function as sexually selected traits. Tail flicking in birds has been shown to be associated with signalling nervousness or alarm (Nero, 1963). Many members of the Rallidae family have contrasting white colouration under their tails, which has been shown to function as an anti-predator signal (Stang & McRae, 2009). Areas

of the body that function to physically communicate particular information can therefore be accentuated by elaborate or contrasting colouration. Insight into the function of conspicuous or contrasting colouration in specific areas may be inferred by the function of that region in communication.

There has been limited research into exploring the spatial organisation of colouration. One recent study considers how colour is arranged on the bodies of birds (Delhey, 2019). This study found that the placement of conspicuous colour on the bodies of birds is non-random in that elaborated colours are most often found ventrally and on the heads of birds. Inconspicuous colour was more common dorsally. Cryptic colouration on the backs of birds camouflages them from aerial predators, whereas conspicuous colour in the front of the body aids in communicating face-on. Birds often face one another when interacting, therefore we can infer that elaborate colouration in the front of the body predominantly functions in conspecific communication. This is a very general approach to using the spatial organisation of colour to infer function when we consider how many aspects there are to conspecific communication. Instead, is it possible to infer which aspect of conspecific communication an area (and therefore colouration in this area) functions in communicating?

To be able to infer the function of different body regions in communication, it is important to consider what information certain species may need to convey. Life-history traits are a way of considering this. Life-history traits are demographic traits that shape the life cycle of different species (Flatt & Heyland, 2011). These traits consist of things like life span, growth rate, age at sexual maturity, clutch size, mating system and so on. For each of these traits, different species have certain strategies that evolve through selection pressures imposed on a species by their social and physical environment (Balasubramaniam & Rotenberry, 2016). Different environmental pressures can for example select the mating system of a species (Heinsohn, 2008), where environments that make it difficult to find a mate can lead to the evolution of a polygamous mating system (M. L. Taylor, Price, & Wedell, 2014). Species that are polygamous need to procure more mates than monogamous species do, therefore traits that help in acquiring a mate (such as plumage colour traits) have stronger selection pressures exerted on them. This is demonstrated by polygynous species generally being more colourful than monogamous species (Andersson, 1982; Moller & Pomiankowski, 1993). Observing where colouration is the most conspicuous on the bodies of species with different life-history traits could therefore potentially elucidate the function of colour traits in different regions of the body. In order to do this, it is essential to be able to directly compare colouration in the different regions of birds' bodies. Birds are highly morphologically variable however as a consequence of environmental adaptation (Snodgrass, 1902), which makes direct comparison of colour difficult. Developing a method that allows for this comparison is therefore essential to studying the spatial organisation of colour in birds.

1.6 Summary and purpose of this thesis

This literature review summarised that there are several knowledge gaps in the bird colouration research area. Particularly, the function of colouration on the specific location on the bodies of birds has not been explored thoroughly. This is an area of research that has been successful when exploring the function of colouration in other taxa and is starting to be considered within avian studies within the last few years. In regards to carotenoid

pigmented colouration, it is evident through this review that ideas concerning the cost of using this pigmentation as an honest signal are being reevaluated. The purpose of this thesis is therefore to explore some of these knowledge gaps.

In this thesis I attempt to answer the following three questions, allocating one thesis chapter each.

1. Is the cost of using carotenoid-pigmented colour in the bills of blackbirds (*Turdus merula*) a social one?
2. Can we develop a novel method that removes the morphological variation in birds that will allow us to examine the spatial organisation of plumage colouration in birds?
3. Are there correlations between different life-history traits and different regions of birds' heads and can these correlations relate back to the function of colour signalling in different body regions?

To research the above-mentioned questions, I will use two different research methods. An experimental approach will be used for researching bill colouration in blackbirds. Experimentally altering bill colouration allows me to study the function and associated cost of elaborate bill colouration. The other two questions are explored using a comparative approach which although correlative, allows for statistical power, due to the availability of large amounts of data (Dale et al., 2015). Comparative studies allow us to look at evolutionary change (Harvey & Pagel, 1991), such as the function of colouration in different groups and body regions of birds. When using both of these methods together it gives a strong research project that has both precision and power. When studying bird colouration, comparative studies are becoming more popular (Freckleton, Harvey, & Pagel, 2002; Mason et al., 2014), as they allow us to include more species whilst being relatively low cost and without being excessively time consuming.

The hypotheses I am going to test for each question are as follows. For carotenoid pigmentation I hypothesise that there is a social cost associated with using this pigment as a badge of status. I predict that individuals with a brighter coloured bill (and therefore higher carotenoid concentration) that intrude into another male's territory are more likely to be aggressive to and receive aggression from brightly coloured territorial males. I predict that the cost of carotenoids is a socially-mediated cost, rather than a resource-mediated cost in the form of carotenoids being limited by diet. For the second question I hypothesise that there is a specific area in birds that is more likely to be colourful than other areas of the body. I expect that areas that contain conspicuous colouration are likely to have a communicative function. I predict that these areas will be in either the chest or head as these are the areas that competitors or potential mates assess when facing each other head on. For the third question I hypothesise that some regions of the head are more likely to be associated with certain life-history traits than are other regions. The life-history traits that I will include in this analysis are migration, cooperative breeding, body mass, wingspan, mating system, paternal care, sexual size dimorphism (SSD), and clutch size. I will also include latitude and seasonality as environmental variables, as these variables are strongly correlated with numerous life-history traits (Pianka, 1970).

I predict that cooperative breeding and migration will select for elaborate colour traits in the crown or the chest, this is due to these variables being associated with social selection and

social interactions often occurring face on. I predict no a priori spatial clumping for correlations with mating system, body size, wingspan, SSD, and paternal care. My rationale for this is that these variables are highly associated with mating system and possibly female choice and that females can presumably assess all plumage traits during longer social interactions involved in mate assessment.

Chapter 2 The cost of carotenoid ornaments: A role for social enforcement?

2.1 Abstract

The honesty of ornamental signals of quality can be maintained by either developmental costs associated with resource availability or social costs associated with aggression. The cost of using carotenoids as a pigment in ornamentation is often argued to be because carotenoids are acquired through the diet and therefore potentially limited in availability. In addition, there are hypothesised trade-offs between using carotenoids for its immunological and antioxidant function versus a pigment. However, the idea that carotenoids are developmentally costly has been recently challenged, and an alternative is that the cost of using this pigment might be a social one. This study aimed to replicate a study using model blackbirds (*Turdus merula* – a species with carotenoid-pigmented bills) to study the response of territorial males to intruder males depending on their bill colour (i.e. high or low carotenoid concentration). The previous study found that intruder male models with bright orange bills received more aggression than did models with dull yellow bills. Here I use an identical experimental framework to explore how this differs when you include the effects of the bill colour of the territorial male. In addition, I also explored the feasibility of using 3D-printed models, compared to taxidermy models, in behavioural studies. My results were inconclusive due to the lack of response from territorial males. My results contrast with the strongly aggressive responses that were received from the males in the study I replicated.

2.2 Introduction

There are diverse functions of communication which include species recognition (Delhey et al., 2007); mate attraction; resource competition; group cohesion; and minimising predation risk (J. A. Endler, 1992). Communication in birds varies between auditory, visual (colour as well as displays) (Marler, 1957), olfactory (Balthazart et al., 2009) and tactile communication (Avilova et al., 2018). Aves are one of the most colourful taxa of animals, making them a common and ideal study taxa for studying visual communication (Shawkey et al., 2009). The huge variation in colouration that exists within and between bird species has arisen due to several different selection pressures acting in concert: natural selection, social selection, and sexual selection (West-Eberhard, 1983). Brightly coloured ornaments are often present due to social selection (Lyon & Montgomerie, 2012), which typically encompasses social competition. These competitions can be over access to resources such as food, territories, and mates (West-Eberhard, 1983). Because interactions during these competitions can become aggressive by way of direct combat, they are potentially very costly to competing rivals (Young, Cain, Svedin, Backwell, & Pryke, 2017).

Signals known as ‘badges of status’ help to avoid escalated agonistic interactions when competing for a resource (Pryke & Andersson, 2003) and reduce costs associated with aggression. These traits are argued to signal the aggressiveness and fighting ability of an individual, as well as the individual’s ability to endure aggression. This means that the winner of a contest can be predicted using their badge of status (Chaine et al., 2011; Rohwer & Rohwer, 1978). Most studies investigating social traits in birds focus on plumage traits, as this is where the majority of elaborate colouration occurs. However, plumage colour only shows the quality of an individual at the time of moult and does not depict the current health of an individual because the colour of plumage is produced during a single seasonal moult (Dey et al., 2017). In contrast, bare parts (such as the bill, legs, and wattles)

are potentially more accurate in representing the current health and vigour of an individual as these parts are vascularised, meaning that pigments are continuously deposited into these areas (Karubian, 2008). Colouration in bare parts, such as in the bill, can therefore be an important signal in social situations, as it can reflect rapid changes in social circumstances (Karubian, 2008; E. S. A. Santos, D. Scheck, & S. Nakagawa, 2011b). Social interactions have the ability to make physiological changes within an individual (such as hormonal and metabolic changes) (Tibbetts, 2014), and condition-dependent elaborate traits can change as a response (Ardia et al., 2010). Dynamic signals can reflect these internal changes in condition, making them ideal badges of status.

An important group of pigments that are argued to reflect the condition of an individual and are often used in bare part signalling are carotenoids. Carotenoids produce yellow, orange, and red colours (Dawson & Bortolotti, 2006), and with bill signalling these pigments are constantly deposited and can therefore be used as a reliable badge of status for the current health of an individual (Biard et al., 2010). Traits that reflect quality are said to have to be costly to be honest signals of quality (Zahavi, 1975). There are two main hypotheses for how honest signalling is maintained: through developmental costs limited by resource availability (Olson & Owens, 1998; Pryke & Andersson, 2003); and through social costs associated with aggression (Maynard Smith & Harper, 1988; Rohwer & Rohwer, 1978). What are the costs that keep carotenoid-based signals honest? The typical argument is that carotenoids are derived from the diet and cannot be synthesised by birds (García-de Blas et al., 2016). The brilliance of carotenoid colours can represent a proxy for the acquisition of this pigment through the diet and consequently can reflect the foraging ability of an individual (García-Navas, Ferrer, & Sanz, 2012). Because carotenoid molecules also function in immune response (McGraw, Nolan, & Crino, 2011) and as antioxidants (Moller et al., 2000), it is costly to use these molecules as pigments rather than for physiological functions (Dawson & Bortolotti, 2006). The ability of an individual to use carotenoids as a pigment therefore reflects their health (Dawson & Bortolotti, 2006), which is further correlated with several traits of vigour such as hunting skill and parental care (Casagrande et al., 2006; Preault, Chastel, Cezilly, & Faivre, 2005). Thus, using carotenoids as a sexually selected signal is said to be kept honest due to the restricted availability of these pigments in the diet (García-de Blas et al., 2016; McGraw et al., 2011) as well as through the trade-off between biological function and pigmentation (Baeta, Faivre, Motreuil, Gaillard, & Moreau, 2008).

Recent research however has argued that carotenoids are not in fact always limiting (Simons, Maia, Leenknegt, & Verhulst, 2014). Species in the previous study that utilise carotenoid-dependent colouration were found to have higher levels of circulating plasma carotenoids than species that do not use carotenoid pigments in signalling. Plasma carotenoid levels were independent of diet which indicates that carotenoid pigmentation is not limited by the acquisition of these pigments. This is further corroborated by the discovery that animals do not favour carotenoid-rich foods and there does not seem to be a limited availability of carotenoids in the environment (Catoni, Peters, & Schaefer, 2008). The presence of circulating carotenoids in birds that do not use these molecules for signalling purposes suggests that there are adequate amounts of this pigment to be used in physiological functions, particularly when considering that the role of carotenoids as antioxidants in birds has been shown to be overvalued (Costantini & Møller, 2008). The previously hypothesised costs of using carotenoids as an honest indicator of quality are therefore under a strong challenge. Recent research further argues that in some cases,

carotenoid-based signals are kept honest through social costs (Dey, Valcu, Kempenaers, & Dale, 2015; Pryke & Andersson, 2003). These studies are consistent with the hypothesis that the cost of using carotenoid pigmentation is the aggression received from other males. Males will act aggressively towards individuals that are displaying a false signal (M. S. Webster, Ligon, & Leighton, 2018) that does not match up with their behaviour (Tibbetts & Izzo, 2010). The cost occurs when receiving aggression from males of higher quality, as opposed to males with similar quality whose aggression can be endured (Pryke, Lawes, et al., 2001; Yasukawa & Bick, 1983).

Because bare-part signals reflect current condition, bare-part ornaments are likely to be important in aggressive and competitive contexts (Dey et al., 2015). Male European blackbirds have carotenoid-pigmented bills that vary in colour from dull yellow to bright orange from the onset of sexual maturation (Preault, Deregnaucourt, Sorci, & Faivre, 2002). A previous study (Bright & Waas, 2002) examined whether the concentration of carotenoids in the bill of intruding male blackbirds affected the response of territorial males. They found that models of intruding males with bright orange bills were approached faster and more aggressively than models with yellow and brown bills, respectively (other studies have found similar results (Dey et al., 2015; Pryke & Andersson, 2003; Pryke, Lawes, et al., 2001)). This suggests that having a bright orange bill is more costly than having a yellow bill due to the increased aggression received from other males. However this study did not investigate the effect of the colouration of the territorial male, and if bill colour signals social status and aggression then this is predicted to additionally influence social interactions. In this study I wanted to explore this idea further by studying the aggression that an intruder male would receive from a territorial male, depending on the bill colour of the territorial male. I predicted that territorial males with orange bills are more likely to be aggressive to intruder males with orange bills, than are territorial males with yellow bills. Territorial males with yellow bills are more likely to ignore the intruder if the intruder has a bill with a higher carotenoid concentration than him (i.e. an orange bill). Individuals of low quality will often avoid agonistic interactions with individuals that possess high quality-reflecting badges of status, as entering into an aggressive interaction with an individual of higher quality is potentially costly (Pryke, Lawes, et al., 2001). Males that possess a badge of status that is similar or higher than an intruding male are more likely to act aggressively as they are more likely to win a contest and therefore maintain access to a certain resource (in this case territories and mates).

The previous study by Bright and Waas (2002) used taxidermy models in their model presentation study. The use of models in studies removes potentially confounding behaviours from treatment individuals (Pryke, Lawes, et al., 2001) - allowing for the isolation of the treatment effect (Crowley & Magrath, 2004). The use of taxidermy models however introduces issues associated with individual trait variability found in natural populations as well as animal ethics concerns (Santos et al., 2011b). This is where the use of artificially produced models can help to avoid these concerns. Using three-dimensionally (3D) printed models in behavioural studies is becoming increasingly popular as a consequence of this (Anderson, Jones, Moscicki, Clotfelter, & Earley, 2016; Bartolini et al., 2016; Behm, Waite, Hsieh, & Helmus, 2018; Igic et al., 2015). To my knowledge however, there have been no studies that assess whether the use of 3D-printed models compares to that of using taxidermy models. A second, separate, objective of my study is therefore to examine the suitability of using 3D-printed models compared to taxidermy models in avian behavioural

studies.

2.3 Methods

2.4 Study site and study species

I carried out this study on Massey University's Ōtehā Rohe campus in Albany, Auckland, New Zealand (-36.734, 174.693), during the months of July, August, and September of 2018. I carried out experiments on days when it was not raining and there was no fog, but all other weather conditions were included in the study. Experiments were performed between the hours of 0730 (sun rise in July) and 1100, as this is when blackbirds are the most active and aggressive (Dabelsteen, 1984).

European blackbirds (*Turdus merula*) are a sexually dichromatic species (Bright & Waas, 2002), which are introduced and widespread in New Zealand (Blackburn, Monroe, Lawson, Cassey, & Ewen, 2013). Blackbirds are an appropriate species to use for this study as they are socially monogamous (Preault et al., 2002) and both sexes defend year-long territories (Snow, 1956). Thirty males have previously been colour-banded on the Auckland Massey University campus, allowing for individual identification. Photographs have been taken of these individuals over a period of one year. The global positioning (GPS) data from these photographs were utilised to determine the approximate territory location and boundaries of the banded resident males. In addition to this, visual surveys were performed to increase the accuracy of territory locations. Pair formation of blackbirds takes place before the month of July (Gurr, 1954), therefore territories should be occupied and defended during this time. All research protocols were carried out under animal ethics consent (Massey University Animal Ethics Consent number: 18/42).

2.5 Model presentation experiment

The methods of the Bright & Waas (2002) study were replicated for this study. Their study found that ultraviolet (UV) reflectance has no effect on the difference in response of males to the models, hence I did not include the effects of UV. I included two model types: 3D-printed and taxidermy. The 3D-printed models had 3 treatment levels: brown coloured bill with juvenile plumage; dull yellow coloured bill with adult male plumage; and bright orange coloured bill with adult male plumage (see Figure 2-1). Each treatment level had two replicates, making a total of six 3D-printed models. The taxidermy model had black plumage with two treatment levels: a dull yellow coloured bill; and a bright orange coloured bill. A single taxidermy mount was used and painted between presentations to change bill colour. The 3D-printed juvenile model was used as a control as juveniles lack the yellow to orange bill colour of mature males and a response towards these models was therefore not expected as they should not be perceived as a threat to territorial males (Studd & Robertson, 1985).

The 3D model template was created by scanning the taxidermy mount, which had a neutral posture (Garcia, 2003), resulting in all models having this posture. Bill colour was manipulated with the use of Resene 'total colour system' paint. The colour of the paint was matched to reflect the natural variation in bill colour that exists within this blackbird population using spectral analyses (see Appendix 1, Table 1). The paints were used on both the 3D-printed and taxidermy models (McNett & Marchetti, 2005). All models were placed on a wooden mount so that they would be visible above grass.

Every territorial male was presented with every level of treatment (five in total) where

presentations were separated by one to two days to prevent habituation (Chaine & Lyon, 2008; Pryke, Lawes, et al., 2001). To control for the effects of responses to each model, presentation order was randomised using a random number generator (http://numbergenerator.org). Models were presented to resident males by placing them on the ground in the approximate centre of each male's territory (Chaine & Lyon, 2008; Mays & Hoppert, 2004) as this is most aggressively defended (Dabelsteen & Pedersen, 1990). I filmed the model presentation for review afterwards (using two GoPro HERO4 cameras). The experiment started when the resident male was approximately ten metres away from the model or within line of sight (if shorter than ten metres due to obstruction from buildings), as this would ensure that the male was within his territory (Snow, 1956). Once within this radius, ten minutes was timed to record the response of the resident male to the model. The observer was positioned twenty metres away to ensure that their presence did not affect the response of the male (Preault et al., 2002).

I recorded behavioural responses in the same way as Bright & Waas (2002): the time a male spent less than two meters, two to ten metres, and more than ten metres within range of the model (as a proxy for a latency to approach (Hick, Doucet, & Mennill, 2015)); and the intensity of display from the territorial male, directed towards the model (see Bright & Waas, 2002). All distances were measured and marked. Additionally I recorded the "time to display" as display should be quicker the more of a threat the model male is (Snow, 1956). To include males that did not respond to the model, males that came within ten metres of the model were considered to have seen the model and to be ignoring it if he did not react to it (Preault et al., 2002; Studd & Robertson, 1985). Model presentations began on the 2nd July 2018 and carried through until 7th September 2018. Males did not respond to the models in July, I therefore decided to stop presentations and resume them in August at the beginning of the egg-laying season. This was because males are predicted to respond more aggressively to intruder males during egg-laying (Creighton, 2001) than during territorial acquisition. Presentations were conducted for approximately 50 – 70 hours in total, most of which was spent with no activity.

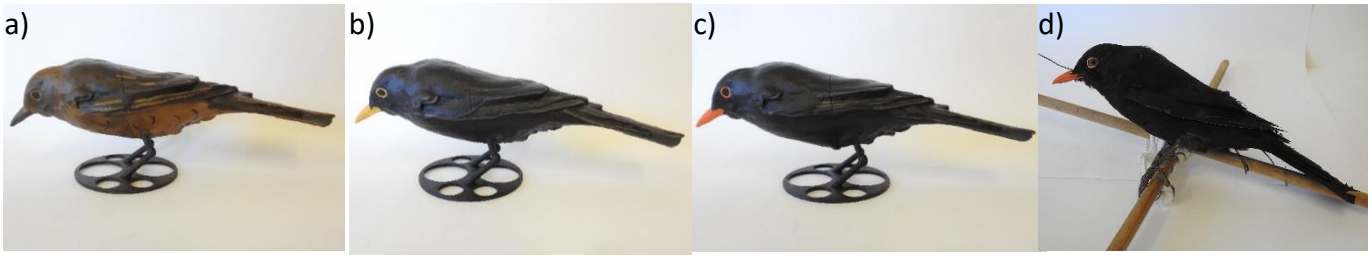

Figure 2-1 Images of all four treatment levels included in this model presentation study, showing both the 3D-printed models: a) brown-billed juvenile model, b) yellow-billed and c) orange-billed male models, and the taxidermy model: d, shown here with bright orange bill.

2.6 Data analysis

I intended to perform a principal component analysis to look at the response intensity and make an overall aggressiveness measure. The colour of the territorial male's bill was to be included in the analysis, to see whether this had an effect on his response to the model (Pryke, Lawes, et al., 2001). Due to the low responses however (see results), I did not have the power to perform the analysis.

2.7 Results

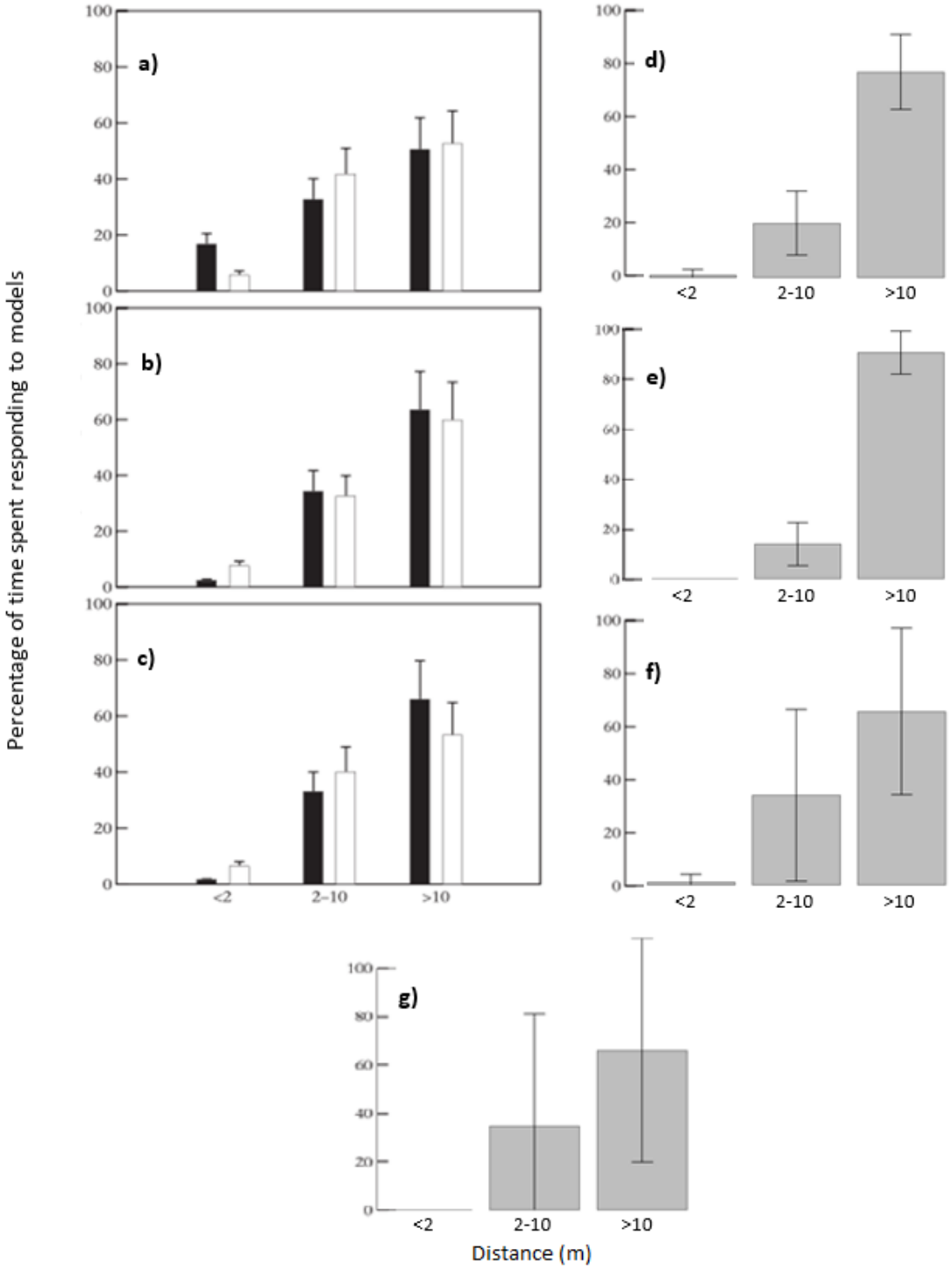

Figure 2-2 A comparison between the results of Bright & Waas' (2002) (a, b, c) and my results (d, e, f, g, n = 8). These graphs show the percentage of time spent by the territorial males at different distances from the various models: orange bill (a, d), yellow bill (b, e), brown bill (c, f) (all of which are 3d-printed models in my study), orange-billed taxidermy model (g).

In total, twenty territorial males were presented with the models. Only eight males responded to the models, some responding to the same models (some models were presented to the same male again in August), thus there were only fifteen recorded "responses". Taxidermy models were shown to the males more than once, in an attempt to elicit a response from the males. There were therefore multiple taxidermy presentations for

some males. Disregarding these, there was a total of eleven recorded responses. This number of responses does not allow us to adequately analyse the behaviour of territorial males. In place of an analysis, I compared my responses to those of the males in the Bright & Waas (2002) study (see Figure 2-2). I was therefore not able to test the effect of the bill colour of the territorial males.

It is important to note that the only "responses" included in these results are the distances that territorial males were found from the models. There were no aggressive responses witnessed, and the strongest response recorded was one alarm call from a territorial pair, and one instance of tail flicking (which can indicate aggression (Bright & Waas, 2002)). The majority of presentations were met with the absence of the male (and female most of the time) from the territory. In cases where the male was absent, I proceeded with model presentation for 30 minutes or more before ending the experiment. In other instances, the male would not come within ten metres of the model, meaning that the ten-minute period never commenced for those males. For the model presentations where the ten-minute period was started, there was a bias towards males approaching the juvenile model. Of all responses, 33.3% responded towards the juvenile model, followed by 26.6% approaching the taxidermy model and 13.3% approaching the yellow-billed 3D-printed model. Once within two metres of the model most males would forage, thus ignoring the model. The only male in my study that came within two metres of the orange billed model had the third most colourful bill of all males included in this study.

2.8 Discussion

Due to limited responses, I cannot draw any conclusions about the cost of carotenoids being potentially a social cost from this study alone. Negative results are still important to report on however, in particular when they contrast with other similar studies. The publishing of negative results has several benefits: it demonstrates that an area of research has been considered (and possibly futile); it prevents future researchers from misusing time and funding to reach the same conclusion; and it can help future researchers rationalise the next step to explore (Weintraub, 2016). It is important however to consider whether the negative results of a study reflect true or false negative results (Taragin, 2019). To confirm a true negative result more data would need to be collected (Hernberg, 1981), however a contrast in results between my study and those of Bright & Waas (2002) can help to elucidate this. Whilst some outcomes were comparable between the studies (such as finding that majority of time was spent further than ten metres away from the model), other findings contrasted greatly.

All of the resident male blackbirds included in the Bright & Waas (2002) study displayed extremely strong responses to the models. The ten-minute timed period in their study begun when the territorial male responded aggressively towards the model, by either "flying towards or vocalizing at the model". This means that all of the territorial males that responded (and therefore were included in the study) did so aggressively. This greatly contrasts with the responses of the territorial males in my study, whose only response was their approach of the models, which also differ between the two studies. The males in their study spent significantly more time within two metres of all models but did so predominantly with orange-billed models. The male blackbirds in my study spent majority of their time in the more-than-ten metre bracket. The males that approached within two metres of a model did so predominantly with the juvenile model. One orange-billed model

was approached within two metres in my study by a male with a duller coloured bill than the model. If proximity is considered to be an important indicator of aggressiveness (Studd & Robertson, 1985), then according to my predictions, juvenile models and orange-billed models should have contrasting responses from territorial males, which they did not. Also, males with dull coloured bills should avoid models with a more colourful bill than them, but this was also not the case. These results therefore contradict my predictions, and contradict the results of Bright and Waas' (2002) study.

The difference in results between this study and the Bright & Waas (2002) study could be due to several factors: my study occurred later in the season, it is therefore possible that the banded males had already acquired territories and therefore were not vigorously defending them. Territory defence is the most aggressive in blackbirds during territory acquisition and during nest building (Snow, 1956) and the period between these times may not elicit as strong a response to territory intrusions. Due to this, model presentations were attempted again during egg-laying, where aggression towards intruder males should be more intense (Creighton, 2001). Testosterone levels increase during this time (Wingfield & Farner, 1978), which is correlated with increased aggression (Schwabl, Wingfield, & Farner, 1985). However aggression towards the models did not increase during this second attempt. A study of the fluctuating levels of testosterone throughout the year in blackbirds could therefore be beneficial in exploring which phase of breeding (territorial defence, nest building, or egg laying) creates higher testosterone levels. This could help to infer when blackbirds are the most aggressive. It is also possible that the difference in levels of aggression could be influenced by the difference in study areas (woodlands (Bright & Waas, 2002) versus suburban campus). Levels of aggression have been shown to be different between rural and urban sites due to factors like population density (Davies & Sewall, 2016) and resource availability (Foltz et al., 2015).

In terms of justifying the use of 3D-printed models in behavioural studies, again the results are inconclusive due to the lack of responses in this study. This is a research area that obliges investigation however, due to the increased use of artificial models in behavioural studies. Three dimensional printing can be an invaluable tool in these studies due to the relative low cost, the ease and time efficiency of printing, and the increasing variety of available materials to print in (Kalsoom, Nesterenko, & Paull, 2018). The suitability of using this method therefore needs to be considered to allow for this advancement in research. I therefore recommend the implementation of such studies.

The purpose of this study was to examine the cost of using carotenoid pigmented colours in socially selected signals. This study unfortunately did not achieve this result, and left more questions than answers due to the low response rate and data. A previous study (Pryke, Lawes, et al., 2001) from another species shows that the carotenoid-based signal colour of the territorial male's plumage can indicate the strength of the response towards intruder males. A study using the bill colour of resident males in blackbirds could help us to determine whether carotenoids are a socially selected signal in bare part ornaments that are kept honest through the aggression of other males. Due to the nature of bare part ornaments reflecting the current condition of an individual and colour thus potentially changing after an agonistic encounter, I expect that this relationship is stronger in bills than for plumage traits.

Chapter 3 Signalling hotspots in passerines resolved with a new method to compare the spatial organisation of body colouration

3.1 Abstract

Studies on colouration in insects and reptiles show that considering the spatial organisation of colour is important. However, research into the adaptive function of colouration in birds has not thoroughly explored the spatial organisation of colour on the body. Here I develop a method that makes it possible to explore the spatial arrangement of colour in birds. The method removes extensive morphological variation that exists amongst birds by warping images of each species into a single reference template. This allows for the direct and comprehensive comparison of colour in all similar patches between species. In order to resolve where signalling hotspots occur on bird's bodies I used sexual dichromatism as a proxy of signalling. That is, I reasoned that the location where a difference in colour between the sexes occurs indicates where signalling is most important in the more elaborate sex. An analysis of 2,471 species of passerines revealed that sexual dichromatism is the most prominent in the chin, supercilium and upper breast. This indicates that these are important physical areas used for signalling in passerines. This finding is most likely due to conspecific interactions often occurring face to face, thus allowing individuals to assess one another during competitive and sexual interactions. The novel method described here also has scope beyond avian colouration studies.

3.2 Introduction

Birds are often studied in regards to the function of colouration because they are one of the most colourful taxa of animals (Delhey et al., 2007). From an evolutionary perspective, elaborated plumage traits are historically of particular interest because they do not offer any obvious survival advantages and often appear to be outright costly to their bearers. Colour traits function to signal several types of information - one of the most common messages that is communicated is the quality of an individual which is important information during mate choice assessment (Askew, 2014). Another function of elaborate colour traits is to communicate the social rank, aggressiveness and thus the competitive ability of an individual (Pryke & Andersson, 2003). Elaborate colours also fulfil roles beyond these, such as for stun displays for predator avoidance, signals of unpalatability, conspecific alarm displays and much more (Baker & Parker, 1979). Considering the multiple outlined functions of colour displays, discerning the purpose of a specific colour trait in a bird is difficult. This difficulty becomes two-fold when we consider the thousands of species of birds that exist, most of which have numerous different colour traits. Creating a method that aids direct comparison of certain elaborate traits is therefore necessary.

In certain taxa, the spatial organisation of colour on their bodies helps to infer the function of that colour in a communication context. Spiny-footed lizards (*Acanthodactylus erythrurus*) for example have a contrasting red colour on their tail and hind legs which becomes white after becoming gravid. This signal is proposed to function in courtship rejection (Cuervo & Belliure, 2013). The location of this colour helps to accentuate the signal as well as to infer its mating-related function because this area is approached and assessed by the male when attempting to mate. Another study demonstrated that the location of colour patterns on the bodies of hoverflies determines whether the species is an accurate mimic of certain wasp species or not (C. H. Taylor, Gilbert, & Reader, 2013). The spatial organisation of colour in hoverflies is therefore important in determining mimic type

(Edmunds, 2000). These examples establish the consideration of spatial aspects in colouration studies in other taxa. Corresponding studies in birds are, up until recently, relatively limited.

A recent study by Miller et al (2018) found that plumage colour in the 230 species of the woodpecker family (Picidae) is non-random. They showed that several variables - like habitat and climate – are strongly correlated with plumage colour and patterning. Phenotypes of sympatric species will often converge (even when accounting for phylogeny) (Miller et al., 2018), exhibiting the strong selection pressure that different environments exert on plumage colouration. We know however that sexual selection (both intra- and intersexual) also exert a force on the plumage colouration of birds. There must therefore be a trade-off between natural and sexual selection, resulting in a predictable pattern of colour across birds' bodies. A recent study by Delhey (2019) considered this trade-off. He found that conspicuous colouration and sexual dichromatism is most common on the front and ventral aspects of the body, with less conspicuous colouration being common dorsally. This finding is intuitive when we consider how birds are perceived by both conspecifics and heterospecifics within their environment. Dorsal body regions are commonly exposed to predators (Gomez & Théry, 2007) and thus cryptic colouration is localised in these areas to avoid predation (Cuthill et al., 2016). Thus, conspicuous colour traits are constrained to ventral aspects. An interesting question is, are specific areas within the ventral region used to signal specific information?

If we consider that the physical location of a signal could greatly increase the reception and perception of a signal by receivers, then certain body regions may be used to signal specific information. For example, wings are often exhibited during mating displays and colourful or contrasting colouration under the wings have been shown to function as sexually selected signals (Jawor & Breitwisch, 2003; Maia et al., 2012). Attention is drawn to the underwing colour by the mechanical movement of the wings, and it is it the colour trait that is used to assess the quality of an individual by the brilliance of the colour. Tail colouration introduces another example where flicking of the tail indicates anxiety or alarm and colouration in this area, particularly contrasting colouration (such as the white colour under the tails of most Rallidae), is used to accentuate this message (Nero, 1963; Stang & McRae, 2009). We can therefore assume that the function of colour in this region is to communicate social information to conspecifics (such as anti-predator alarm signals (Stang & McRae, 2009)), even without the physical movement of the tail. These examples indicate that specific regions of the body are often associated with communicating specific information. We can therefore gain clues to the purpose of a specific colour trait by considering where the trait is placed spatially on the body of the bird.

To resolve the spatial organisation of colour we need to resolve which parts of birds' bodies typically and consistently display elaborated colouration. Areas that are consistently elaborately coloured across difference species (i.e. hotspots) suggest a relative importance of these areas in signalling because they have been consistently selected to be elaborated. To determine where these hotspots occur, it is essential to be able to directly compare homologous regions of the body of birds. This is a difficult task due to the extreme morphological variability of avian taxa. One way to do this is to manually measure many different patches on as many different species as possible. Delhey (2019) did this using UV-Vis spectrometry across 538 species of Australian terrestrial birds. However, comparing homologous regions in such a manner becomes an arduous task that is time consuming and

introduces error due to human perception of categorising these regions and the limited number of patches one can realistically measure manually. An alternative approach to dealing with morphological variation in relation to traits such as colour is to remove anatomical shape variation entirely (Adams & Otarola-Castillo, 2013) using images of birds that are warped to a common shape. This allows one to focus only on colour variation, and to directly compare all homologous regions across numerous species. The objective of this study was to develop a methodology to remove morphological variation between species. I did this using landmark registration on field guide images to warp the images to a common template.

As a case study to explore which body regions are utilised most for signalling I additionally investigate where on bird bodies sexual dichromatism is most pronounced. To do this I used the difference in colour between sexually dichromatic species as a proxy of elaborated colouration. In the vast majority of bird species, when there are colour differences between males and females, it is the male that is more elaborated (Dale et al., 2015). In many of these cases, sexual dichromatism is argued to result from an evolutionary pressure on the plumage of the female to be dull coloured (Martin & Badyaev, 1996). This further suggests that the specific areas that are elaborately coloured in the more colourful sex (i.e. predominantly the male) are likely to be important in signalling in intraspecific social interactions. Due to the nature of social interactions primarily being carried out face-to-face (Crook, 1960; Popp, Ficken, & Weise, 1990) I predict that colour hotspots will occur at the front of the body (specifically the head and chest). These areas, unlike dorsal regions, are constantly exposed to rivals and potential mates during behavioural interactions. A key distinction between this study and Delhey's (2019) study is that through the use of my novel method trends in colour hotspots are able to be resolved to the pixel level and therefore for the entire body of the birds.

3.3 Methods

3.4 Sourcing the images

Images of each species were sourced from Handbook of the Birds of the World (HBW) (del Hoyo et al., 2002). Birds have vision that is different from that of humans in that they are tetrachromatic. Birds see in four different colour channels, three of these similar to human vision in its entirety and an additional cone providing vision in the ultraviolet (UV) spectrum (Bowmaker et al., 1997). Bergeron and Fuller (2018) have validated the use of human-based vision as a suitable proxy for avian vision in colour oriented studies. They demonstrate that images from field guides can be used as an accurate depiction of the colour of that species. The method of using illustrations in bird colouration studies has also been validated by various other research groups (e.g. Dale et al., 2015; Miller et al., 2018).

The HBW provide illustrations of 9,873 documented species of birds that exist throughout the world, illustrating both sexes for dichromatic species. This study focuses on 2,471 species of passerines for which there is complete life-history trait (LHT) data for size dimorphism, mating system, sociality and various other variables. These data were used by Dale et al (2015) and are to be used in chapter four of this thesis in a comparative analysis looking at correlations between LHTs and different body regions, hence the inclusion of these particular species in this study. The illustrations were digitally scanned using a Fuji Xerox AspeosPort-IV C5575 scanner with default settings (Dale et al., 2015). Each species was represented by one male and one female image. To maintain similar amounts of

measuring error between monochromatic and dichromatic species, images of monochromatic species were measured twice to represent both sexes. For species that have different polymorphs, the most common morph was used. The images were cropped and rotated to all face the same way.

3.5 Image processing

Image registration and warping was conducted within the statistical software 'R' (version 3.5.1) (R Core Team, 2017). The packages 'imager' (Barthelme, 2018) and 'sp' (Pebesma, Bivand, & Gomez-Rubio, 2013) were used in tandem with code (written by J. Dale & D. Playne and myself, see Appendix 2). The code creates a reference template (see Figure 3-1) representing pre-determined body regions (patches) within a bird, each designated by a triangular mesh. These regions were: lower bill, upper bill, lore, chin, auricle, crown, eye stripe, eye, nape, mantle, median coverts, greater coverts, primaries, secondaries, rump, tail, vent, belly, lower breast, upper breast, and legs. Each image of each male and female was processed manually by marking out (registering) landmarks of homologous points. If one of these regions were not entirely visible due to the way that the bird was depicted, for example the rump may be covered by the feather, a region with the corresponding colour would be marked out instead (to do this I used the description of colours in the text of HBW). The coordinates for these landmarks were updated constantly into a dynamic data file. These coordinates outline the 21 polygons that define each body region and the 130 triangles that compose the overall mesh that occurs within the whole-body template.

Once all landmarks were registered the original image was warped into the universal reference template using affine transformations of each of the triangles in each body region (Bartolini et al., 2016; Glasbey & Mardia, 1998). Affine transformations retain the relative positions of the coordinates to each other when warping the image, thereby maintaining relationships between colours in the homologous regions of the birds. This produces images of morphologically identical looking birds (see Figure 3-2), allowing for the identification of exact homologous regions and a comparison of colour in these areas down to the individual pixel level. The colour of each pixel was represented by RGB (red, green, blue) colour values, this colour system is common in most computer monitors (Abdolmaleky et al., 2017). In RGB colour space, each colour channel is represented on a scale of 0 to 255, where 0 is no reflection of that colour and 255 is full reflection. Different levels of these colours therefore combine to create the full gamut of different colours (visible to humans).

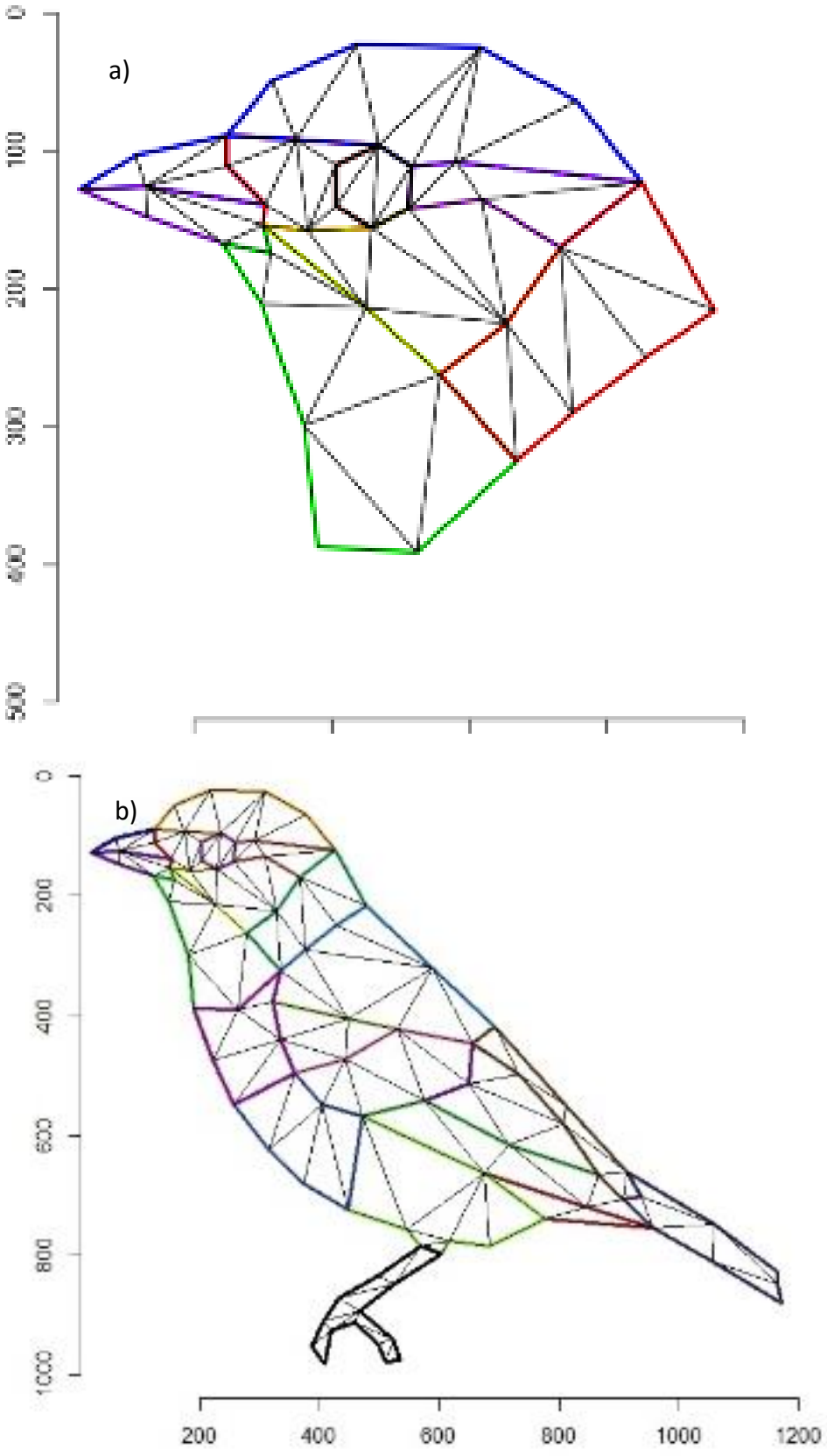

Figure 3-1 Reference templates that are used to remove morphological variation between species and create morphologically identical birds. This allows the comparison of colouration in homologous regions of the head (a) and the body (b) of birds. Illustrations of all species included in this study were warped into these reference templates and colour was compared between different regions of the bird (represented by the triangles) down to the individual pixel level by use of pixel coordinates (represented by axes).

Because colouration in the head region of birds can appear to be particularly complex, two separate reference templates were used during image registration (Figure 3-1) representing the head-only and the rest-of-the-body. The increased detail present in the heads of the images (auricle, eye etc.) required larger sized images for registration. This was not necessary for the bodies however as body regions are generally less detailed. Bodies were therefore registered separately from the heads and were later warped together into one image. The dimensions of the head images are 500 by 500 pixels and the entire body image dimensions are 1200 pixels wide by 1000 pixels high. The head template was used initially in a pilot study to warp the 283 existing species of tanagers. The tanager family (Thraupidae) was used because it displays a huge variation in both colouration and life-history traits (Mason et al., 2014). Due to the success of this pilot study to provide proof of concept and finding a relationship (in the form of colour hotspots) we then increased the number of species to include the heads of 2,471 species of passerines with complete life-history trait data. After this we also included the entire bodies of a subsample of species in order to perform an analysis on the entire body. For the entire body 1,231 species of birds were included, these species were a subset of the original 2,471 species but due to time constraints only roughly half of those initial images were able to be registered. The resulting number of images for 2,471 species is 4,942 (male and female). Processing the images took at total of about 3 months when considering the manual selection of landmarks as well as the warping of images.

Figure 3-2 A visual example of how species' heads (a) and bodies (b) appear morphologically identical after the images have been warped into the reference template using the outlined method. This demonstrates how the colour between these species can now be directly compared when morphological variation is removed (illustrations sourced from del Hoyo, Elliot, & Sargatal, 2002).

3.6 Sexual dichromatism hotspot analysis

I determined the exact difference in colouration between the sexes of each species using the warped images. To do this I transformed the images into matrices, where each matrix represented the colour of an image by reflecting the RGB values of each pixel within the image. For each pixel the Euclidean distance in RGB space between the male and female value was calculated and a difference matrix was created for each species (see Figure 4-3). Each species thus has a single difference matrix, and I then averaged the difference matrices to produce an overall average dichromatism difference matrix. I converted this matrix into an image and used colour scales (blue to red, where red is high and blue is low) to represent the average Euclidean distance between the colours of the sexes at each pixel. This image provides a descriptive quantification that highlights where dichromatism is more pronounced. The code for this analysis is in Appendix 2 section 3. For this analysis I determined six difference matrices: 1) heads of all species (n = 2,471); 2) heads of dichromatic species only (n = 1,161); 3) bodies of all tanager species (n = 283); 4) bodies of dichromatic tanager species only (n = 142); 5) bodies of all species (n = 1,231); 6) bodies of dichromatic species only (n = 577) (see Figure 3-4, 3-5, and 3-6). The tanager species were included in the analyses but an additional analysis of tanager-species-only was done to determine whether the different life-history traits that are characteristic of tanagers affected where colour hotspots occur on their bodies. Many tanager species exhibit female-only incubation for example (del Hoyo et al., 2002) which can indicate whether life-history traits can mediate colour on a spatial scale (an idea that is developed further in Chapter 4).

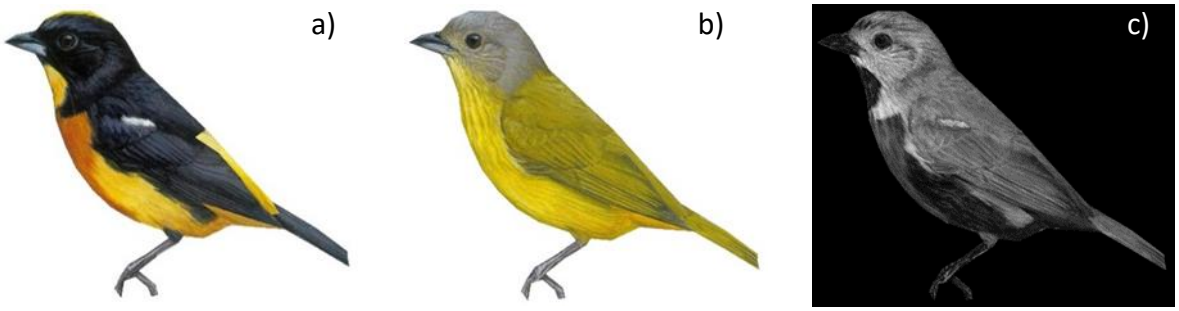

Figure 3-3 An example of how using the outlined method makes it possible to create a summary image (c) of the difference in colour between the sexes of a species (in this case the yellow-crested tanager (Tachyphonus rufiventer), showing the male (a) and female (b)), where white colouration in the summary image indicates a larger difference in colour between the sexes (illustrations sourced from del Hoyo et al., 2002).

Because landmark registration has an element of subjectivity, there are slight differences in the location of the landmarks between images of the same bird (as in the male and female labelled versions of the same image in monochromatic species). These monochromatic species thus introduce artefacts into the analysis because the sexes are represented by the same image and therefore the same image is warped twice – thus will create relatively slight but false dichromatism, particularly along the margins of the different body regions or along the edges of high contrasting plumage colours. Including the monochromatic species within the analysis for the average bird shows the error created by this method (Figure 3-4a, 3-5a & 3-6a). To control for this I performed an analysis with and without monochromatic species. This highlights the biologically real dichromatism and dichromatism plus artefacts respectively, when also including monochromatic species. It is important to note that the analysis here is purely a descriptive one as no phylogenetic or quantitative comparison is conducted. However due to the exploratory and methods development nature of this study,

looking at where colour hotspots occur in sexually dichromatic species in order to test the outlined methodology is valid.

3.7 Results

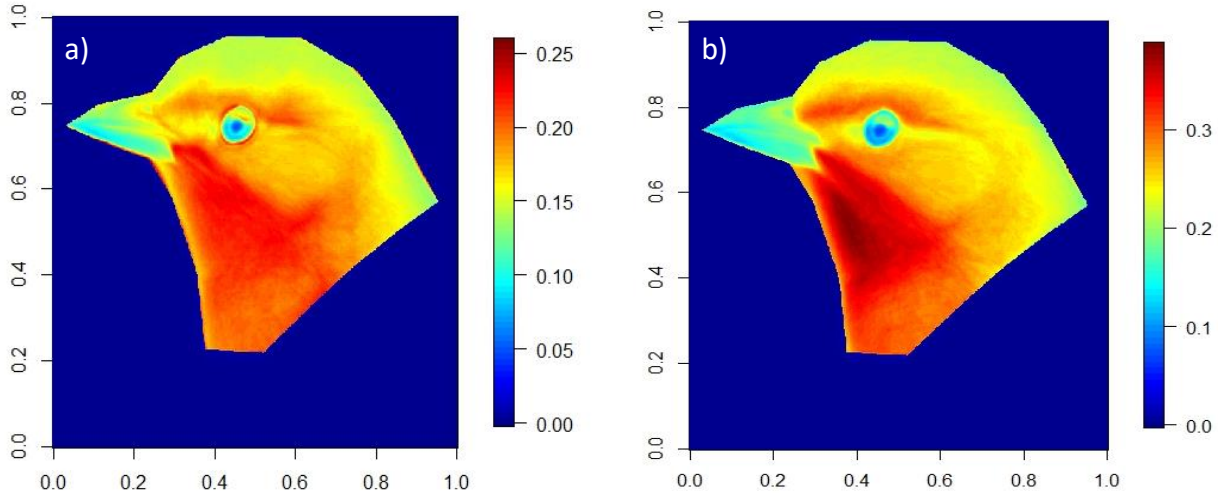

Figure 3-4 Heat map images of the average difference in sexually dichromatic colour in the heads of passerine species of birds. Red colour indicates a high Euclidean difference in colouration between the sexes and blue indicates a low difference (as indicated by the legend). Image a) is created using 2,471 species of passerine birds where both sexually dichromatic and monochromatic species are included, whereas image b) represents dichromatic species only (n = 1,161).

The sexual dichromatism heat map of the heads of all 2,471 species of passerines shows that dichromatism is the most pronounced in the chin region. As expected, this relationship was shown to be stronger when monochromatic species were removed from the analysis (as seen in Figure 3-4b). The high dichromatism index is strongest in the centre of the chin, and this is almost certainly due to artist impressions where shading is included on the left margin, therefore creating a region of lower amount of dichromatism between the illustrations. The deep red chin patch therefore represents the front of the bird rather than the side, as suggested by the image.

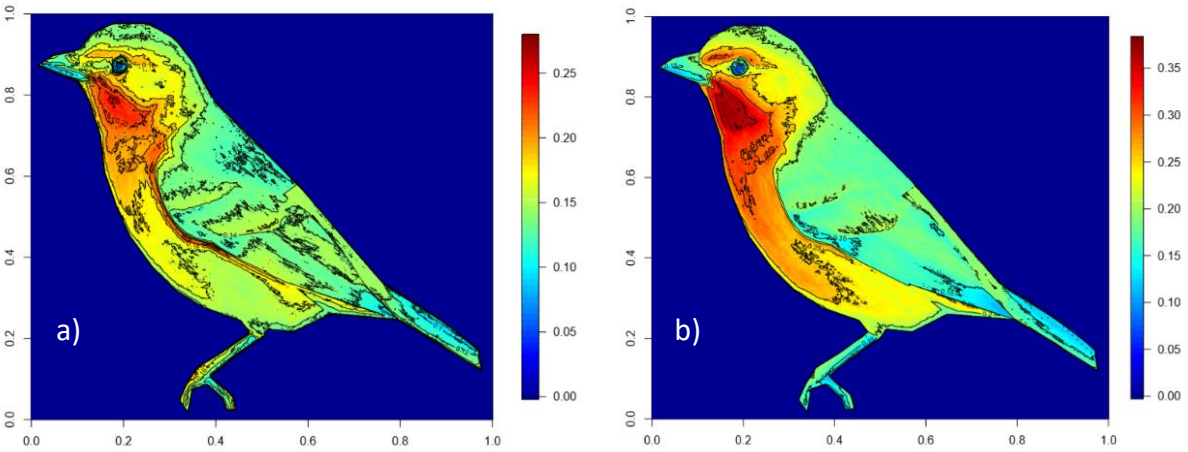

Figure 3-5 Heat map images of the average difference in sexually dichromatic colour in the bodies of passerine species of birds. Red colour indicates a high Euclidean distance difference in colouration between the sexes and blue indicates a low difference (as indicated by the legend). Contour lines were included in this analysis to highlight differences in colour. Image a) is created using 1,231 species of passerine birds where both sexually dichromatic and monochromatic species are included, whereas image b) represents dichromatic species only (n = 577).

The dichromatism heat maps for the whole bodies of all species shows a similar trend, with the chin having the strongest difference in colour between the sexes. When removing monochromatic species, the Euclidean distance between colours in the supercilium increases (Figure 3-5b). One interesting thing to note is the difference in Euclidean distance between the analysis for all species and tanagers only. Tanagers have a much higher difference in colour between the sexes in the back region (nape, mantle and rump, as well as the coverts, see Figure 3-6) than do the other species. This indicates that sexual dichromatism is higher in the tanager species. For both analyses, when monochromatic species are included in the analysis, there is high dichromatism along the wing margin, this is most likely an artefact as this relationship is removed when monochromatic species are removed.

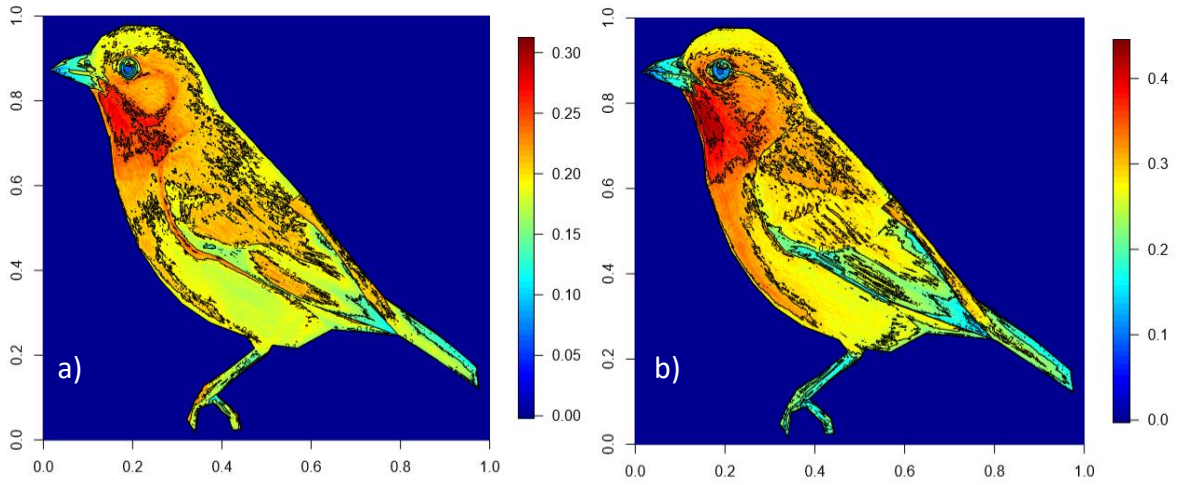

Figure 3-6 Heat map images looking at the average difference in sexually dichromatic colour in the bodies of tanager species of birds. Red colour indicates a high Euclidean difference in colouration between the sexes and blue indicates a low difference (as indicated by the legend). Contour lines were included in this analysis to highlight differences in colour. Image a) is created using 283 species where both sexually dichromatic and monochromatic species are included, whereas image b) represents dichromatic species only (n = 142).

3.8 Discussion

Here I developed a method that permits a detailed spatial exploration of avian colouration. I performed an analysis on a large sample of passerine species, and this has provided, for the first time, a highly resolved image of variation in sexual dichromatism across a broad diversity of species. By using landmark registration and affine transformation, I was able to remove morphological variation among species and perform a direct comparison of colouration between species. This study showed that colour elaboration hotspots are most pronounced on the chin, supercilium and upper breast of passerine birds. This held true for all analyses (heads only, bodies of all passerines and bodies of tanager species only). These findings are similar to those of Delhey's (2019), but on a larger, more comprehensive scale. This facilitated resolving the supercilium to be an additional region of colour elaboration and thus of signalling importance.

Areas of ornamental colouration that occur when considering the difference in colouration between the sexes are most likely due to elaborate colouration in the male. Because females are often the sex that are under selection to be cryptically or dull coloured, this suggests that these areas are important for signalling in males. Indeed in many single-

species studies, there is evidence that the chin is used for signalling social status (Rohwer & Rohwer, 1978) and sexual quality (Johnsen, Andersson, Ornborg, & Lifjeld, 1998). The supercilium has also been shown to function in social contests (González-García, Lara, Quesada, Chávez-Zichinelli, & Serrano-Meneses, 2018). This supports my prediction that the anterior region of the bird would have conspicuous colouration, most likely used for signalling, as these areas are particularly visible to conspecifics during competition or sexual contests (Crook, 1960; Popp et al., 1990).

The all-passerine analysis showed that there is low sexual dichromatism in the dorsal region of birds. Sexual dichromatism is often argued to evolve due to selection for dull coloured females to avoid predation whilst in the nest incubating (Dunn et al., 2015). If so, then we would expect a high level of dichromatism on the dorsal region above the nesting line of birds. However, as shown by the heat map, the back (tail, rump, mantle, and even wings) is relatively cool coloured, indicating a considerably lower degree of difference in plumage colour between the sexes. A problem with the above argument however is that in many species male and females both incubate the eggs, which suggests that both sexes should have cryptic colouration above the nest line. Interestingly, the 44 species of tanagers that were included in this study and have incubation behaviour data display female-only incubation in all species but one (del Hoyo et al., 2002). When the analysis was restricted to the tanager-only heat map the dorsal colour difference is higher than for all species, indicating that males are considerably more elaborate dorsally than are females for tanager species. These results therefore corroborate that sexual dichromatism is more pronounced (particularly on the back) in species where it is the female alone who is the main incubator of eggs in the nest (Martin & Badyaev, 1996).

The non-random organisation of colour on the bodies of birds indicates that these areas are consistently selected upon to conspicuously signal specific information. Signalling of certain information should be conveyed by a specific signal type (or body region) in order for the transfer of information to be clear, thus avoiding costs in signal failure and signal production (Cooney et al., 2019). Therefore the finding that colour hotspots are concentrated on the heads of birds in tandem with the knowledge that birds face one another during social and sexual interactions strengthens the idea that the placement of colour traits in this region is non-random. Conspicuous colour present in the head and chin region of birds permits conspecific individuals to assess and procure information about the quality or competitive ability of another individual. To explore whether specific regions function specifically in signalling a certain information type (i.e. whether the chin always functions in dominance signalling) correlational studies between life-history trait data and specific body regions can potentially be utilised. This is an idea that is further expanded and analysed in chapter four of this thesis.

The study by Delhey (2019) found that rump colouration was an area that exhibited conspicuous colouration when considering both sexes. My analysis shows low conspicuousness in the rump due to a low difference in colouration between the sexes in this region. This is an example of where using sexual dichromatism as a proxy for exploring spatial organisation of colouration in birds may fall short as areas of conspicuous colouration that occur in both sexes are not sexually dichromatic and are therefore not reflected by bright colouration in my analyses. Nevertheless, the inclusion of thousands of species within my study makes this a statistically powerful method.

The landmark registration performed in this study succeeded in collecting morphological variation data for the heads and bodies of 2,471 and 1,231 species of passerines, respectively. This data could be used for morphometric studies. Morphometry is a dynamic field of study (Foster, Podos, & Hendry, 2008) and my method could potentially be incorporated. In conclusion, this study has produced a novel and powerful way of comparing colouration in birds that has scope beyond this taxa and field of study. I have also presented powerful data on the spatial organisation of colour in passerines that can and will be further elaborated on in the following chapter.

Chapter 4 Avian life-history traits as predictors of signalling functions for different body regions in birds.

4.1 Abstract

Elaborate colouration in birds has been shown to be non-randomly distributed on their bodies. This has been argued to be due to trade-offs between conspicuousness used for communication and camouflage used for predator evasion. These patterns suggest that both environmental pressures and social pressures exert selection on the spatial organisation of colour on the bodies of birds. Indeed, intraspecific work has demonstrated that certain areas of the body function to communicate specific information, such as the wings communicating sexual quality (*Cardinalis cardinalis*), the chin communicating social rank (*Zonotrichia atricapilla*), and the tail communicating alarm or nervousness (Rallidae family). This indicates that different body regions may be selected to communicate different types of information. This idea has not yet been explored using an interspecific comparative approach. In this study, I tested whether different regions of the anterior region of songbirds are correlated differentially with evolutionary drivers of ornamental colouration. Specifically I tested the correlations between different regions in the heads of 2,471 species of passerines and eight different life-history traits. My results confirm previous research and show that life-history traits like body size, cooperative breeding, sexual selection, and tropical life history can predict colouration in males and females. Looking for spatial patterns in the correlations of these traits, I did not resolve obvious differential patterns of colouration across the heads of birds. My results did show that bill colour in larger species is more elaborated, and that species that are cooperative breeders and live in tropical regions are more likely to have elaborately coloured irises.

4.2 Introduction

The spatial organisation of colouration on the bodies of birds can help us to understand the function of colourful plumage because different physical areas of birds' bodies can be associated with different kinds of signalling. For example, tail flicking helps to signal alarm (Nero, 1963) and contrasting colouration in the tail area enhances the efficiency of communication of this message (Stang & McRae, 2009). Similarly, colourful or contrasting colouration under the wings have been shown to function as a sexually selected trait (Jawor & Breitwisch, 2003; Maia et al., 2012) which are exposed during wing displays associated with mating. These two examples demonstrate that colouration in body regions used in physical displays often have a similar signalling function to that of the mechanical display. The occurrence of certain information being conveyed by specific body regions might increase the likelihood of the message being received and interpreted by the signal recipient and might provide better opportunities to use different regions to signal different messages. This ultimately avoids costs associated with signal failure and signal production (Cooney et al., 2019).

Conspicuous colour traits have been shown to be non-randomly distributed across the bodies of birds (Delhey, 2019). These traits are most evident in the head and ventral aspects of birds, whereas cryptic colouration is more common dorsally. Additionally , sexually dichromatic regions of birds are spatially clumped, with the throat having the most pronounced differences between males and females (Chapter 3). Elaborate colouration consistently occurring in certain areas of the body suggests that these areas play a differentially important role in signalling different kinds of information. I hypothesise that

due to ventral and anterior regions often being exposed to conspecifics during competitions and sexual interactions, they may function in conspecific social signalling. Conspecific social signalling consists of conveying several types of information, such as: sexual quality or competitive quality (J. A. Endler, 1992). To test this, in this study I evaluate whether elaborate social and sexual traits are more pronounced in specific areas of the heads of birds (specifically within the ventral region as suggested by previous studies).

One way of considering the spatial organisation of signalling is by looking at life-history traits and how they correlate with plumage traits in different regions. It is known that certain life-history traits exert different selection pressures on the plumage of birds. Life-history traits are demographic traits that shape the life cycle of the individuals of a species (Flatt & Heyland, 2011). Examples of these are mating system, clutch size, and parental care strategies. These strategies will evolve due to selection pressures from both the social and physical environment, resulting in the most optimised life-history strategy for that species (van Noordwijk & de Jong, 1986). Therefore different life-history traits could selectively favour signalling in specific spatial locations.

The above rationale provides insight for understanding interspecific variation in plumage colouration. For example, polygynous species are more ornamented than monogamous species due to increased selection pressures of female choice and intrasexual competition for mates (Andersson, 1982; Moller & Pomiankowski, 1993). Badges of status (socially selected traits used in intrasexual competitions to assess other individuals) are more common in group living species due to increased competition (Chaine, Shizuka, Block, Zhang, & Lyon, 2018). Variation in sexual dichromatism and plumage colouration have been shown to be correlated to different life-history traits such as body size, breeding altitude, and migration (Badyaev, 1997). The degree of extra-pair paternity is related to male colour elaboration (Moller & Birkhead, 1994); nest location selects on plumage colouration (Olson & Owens, 2005); and colour patches are the result of increased territoriality in new world blackbirds (Johnson & Lanyon, 2000). These examples demonstrate that life-history trait strategies exert a selection pressure on plumage colouration.

How do different kinds of colour-based signals coexist on the plumage of birds? The aim of this study is to explore where, on the bodies of birds, different types of signalling occurs. I test this by using different life-history trait data and calculating correlations between them and plumage colour elaboration across different regions of the body. My reasoning is that if life-history traits select for communication in certain body regions, then life-history traits should correlate differentially with elaborate colouration across different locations.

In chapter three I developed a method that allows for the compartmentalisation of colour into different regions of the body of birds. Here I follow up on this work to analyse the correlations between these regions and the life-history traits included in this study. These traits are variables that were used in a study by Dale et al. (2015) and are: migration, cooperative breeding, mating system, paternal care, clutch size, body mass, sexual size dimorphism (SSD), and wingspan. I will also include the environmental variables latitude and seasonality as these characteristics are strongly associated with numerous life-history traits (e.g. fast (r) breeding versus slow (k) breeding in temperate versus tropical regions respectively (Pianka, 1970)).

Dale et al.'s (2015) study found that majority of the above life-history traits and variables

can predict the presence of elaborate colouration in both males and females (across 6000 species of passerine birds). My study will contribute to the analysis from Dale et al. (2015) by looking at the spatial arrangement of this colouration. I predict that certain regions of the body will function in signalling certain types of information and therefore that there will be correlations with different life-history traits and different body regions.

4.3 Methods

4.4 Predictions

Based on the hypothesis that specific areas on birds will be more strongly related to signalling associated with different life-history traits I predict that correlates of increased social selection (such as cooperative breeding and migratory species) are more likely to be related to plumage occurring on the front of the bird. This prediction is based on the idea that competitive interactions tend to occur face on (Crook, 1960; Popp et al., 1990). Migratory species have an increased selection pressure to signal competitively when they arrive at their breeding grounds to acquire territory, as well as increased competition for resources in feeding grounds often due to large population densities in these areas (Simpson, Johnson, & Murphy, 2015). Specifically I expect correlations with these traits to be highest in the crown or the chest, as these locations are visible to challengers and can act as a badge of status to help avoid escalation to costly conflict (Pryke & Andersson, 2003).

Because mating systems are associated with strong female choice (Emlen & Oring, 1977), I predict that regions that can be assessed by females are more likely to be elaborately coloured in polygynous than in monogamous species. Specifically I expect these elaborate traits are likely to be placed elsewhere on the head (not just ventral aspects), because females are able to assess more easily the complete plumage patterns of a potential mate. I predict the same to be true for body mass, wingspan (M. S. Webster, 1992), and SSD (Dale et al., 2007) as large body size and male-biased SSD are strongly correlated with polygynous mating systems. Additionally, for paternal care, I predict the same to be true as females can assess males for their parental care ability (Preault et al., 2005). Indeed, sexually selected traits can be found all over the body (such as the wings (Jawor & Breitwisch, 2003) and tail (Gomez & Théry, 2007)) due to members of the opposite sex being able to assess an individual from several angles (not just front-on).

Clutch size, latitude, and seasonality are highly correlated variables. Seasonality and latitude are associated due to habitats at lower latitudes being less seasonal than habitats in northern or high latitudes (Jetz & Rubenstein, 2011). Additionally, species that occur at lower latitudes are known to lay smaller clutch sizes (Lack, 1947). Species in the tropics (therefore species that occur in low altitude, low seasonal environments and have small clutch sizes) are known to be more colourful than species that are found in temperate habitats (Dale et al., 2015). I therefore predict that these species will be more colourful, however, I make no a priori prediction that there will be specific spatial arrangement of colouration related to these traits.

4.5 Life-history trait data collection

The illustrations used for this study were collected as in chapter three. Additionally, the method created in chapter three was further modified for this study. Briefly, this method creates a reference template for the head region (see Figure 4-1). This template is comprised of nince polygons that define each region of the head, and in total contain 54

triangles that compose the mesh. I warped each image of the head region of songbirds into this common reference template. Only the heads of each species of both sexes of the 2,471 where there is complete life-history and trait data are included in this analysis. Life-history traits that were considered in this study are: migration, cooperative breeding, mating system, paternal care, clutch size, body mass, SSD, and wingspan. The environmental variables are: latitude, and seasonality. These variables were taken from Dale et al. (2015) and the traits were scored in the following way (see Dale et al. (2015) for more detail): **migration**: 0 – resident, 1 – partial migration, 2 – complete migration; **cooperative breeding**: 0 – absent, 0.5 – suspected, 1 – present; **mating system**: 0 – strict social monogamy, 1 – monogamy with infrequent instances of polygyny observed, 2 – mostly social monogamy with regular occurrences of facultative social polygyny, 3 – obligate resource defence polygyny; **paternal care**: 0 – absent, 1 – present; **SSD**: log(male wing length) – log(female wing length); **clutch size**: average clutch size calculated from statistics in literature; **latitude**: given as the latitude of the breeding range of a species; **seasonality:** computed from Moderate Resolution Imaging Spectroradiometer land surface temperature rasters, these files were superimposed and an average coefficient of variation was calculated for the temperature in each pixel of the breeding range of a species (spanning 12 years of temperature data); **body mass** and **wingspan** data was averaged from figures previously collated from other sources.

4.6 Statistical analysis for comparative analysis

The program 'R' version 3.5.1 (R Core Team, 2017) and the packages 'imager' (Barthelme, 2018) and 'sp' (Pebesma et al., 2013) in tandem with in-house code (see Appendix 2) were used to analyse the data. The RGB (red, green, blue) values of the images were averaged across all pixels for each of the mesh-triangles in the reference template of each sex for each species. As a measure of plumage elaboration, the distance in RGB space of each triangle for any image was calculated from the average of that triangle across all images. This tends to result in higher values for bright or high contrast colours (such as red, blue and black) and lower values for drab colours (such as olive or brown). Distance calculated in this way correlates very strongly with "maleness" plumage scores used in Dale et al. (2015) (R^2 = 0.78, J.Dale unpublished data).

Thus for each triangle (representing a specific location on the bird) the response variable was colour distance from the average. Correlations were then calculated between each life-history trait and average colour in each of the triangles of the head, for males and females separately. This was initially done for all ten variables separately (see Appendix 3, Figure 1 for these results). Due to many of these variables being intercorrelated they were consolidated in a similar way to what was done in Dale et al. (2015). Body mass and wingspan were incorporated into a body size variable; latitude, seasonality and clutch size were amalgamated into a 'tropical life history' variable where high values represent species that live at low latitudes, with low seasonality and have small clutch sizes; SSD, mating system and paternal care were incorporated into a sexual selection index, where high values indicate social polygyny, male-biased SSD and female-only parental care; migration and cooperative breeding were left as single variables. A multi-predictor model was then performed using these composite variables (Dey et al., 2014).

Another consideration in comparative studies is the non-independence of species. Due to shared ancestry between species, they cannot be used as statistically independent data

points (Bodey et al., 2018). This was controlled for in this analysis by performing a phylogenetic generalised least squares (PGLS). The analysis output provided effect sizes and p-values as a measure of the correlation between each triangle and the life-history traits (see Appendix 3, Table 1 & 2). These p-values are represented by different colours in the summary images below (see Figure 4-2). Significant positive correlations are represented by red, significant negative correlations by blue, and non-significance by white. Darker shades of these colours represent more significant correlations. A significant positive correlation indicates colour elaboration in the region of the body represented by a triangle.

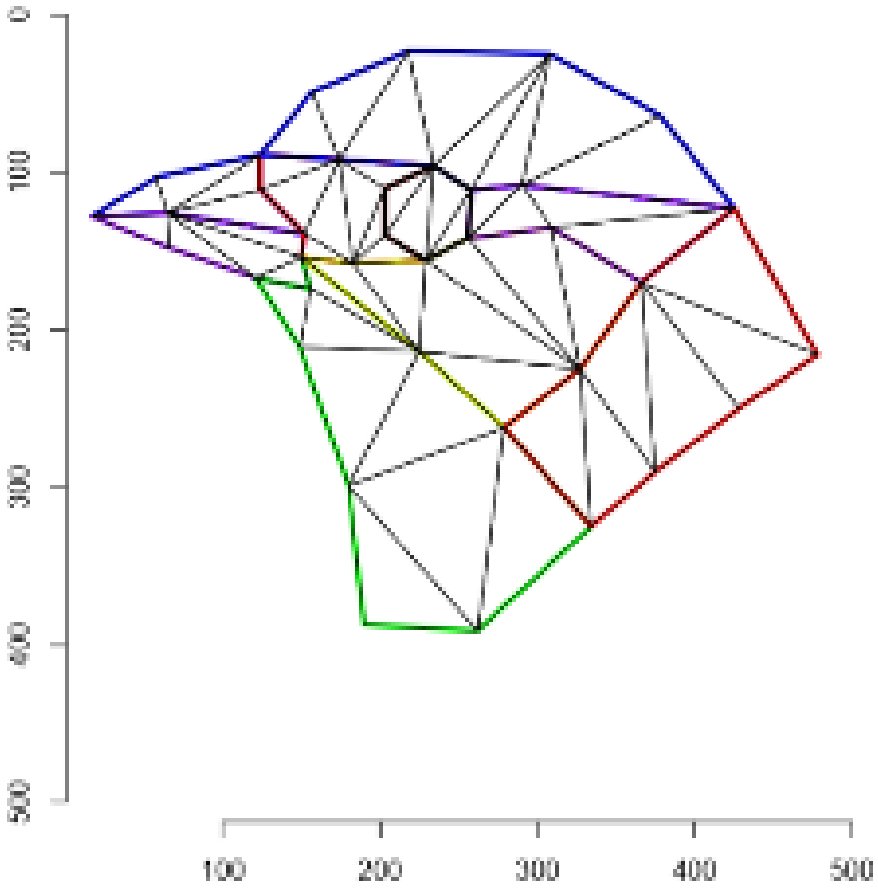

Figure 4-1 Reference template for the head of birds. Images of both sexes of each species are warped into this template, thus removing morphological variation between species and creating species that appear morphologically similar. The triangles in this template represent different body regions, this allows for correlation calculations to be calculated between these triangles and the different life-history trait data included in this study.

4.7 Results

The results indicate that effect sizes for correlations between colour elaboration and certain variables are higher for some variables in single predictor models (see Appendix 3, Figure 1) than when controlling for confounds in a multi-predictor model. For example, in the single predictor model for paternal care, females in species with paternal care are more likely to be elaborately coloured. When we consider paternal care within the multiple predictor model however (see Figure 4-2), we see the reverse of this relationship, where an absence of paternal care results in drab females. This demonstrates that in species where there is high sexual selection on males, females are more likely to be drab coloured. This

demonstrates the importance of the multiple predictor model in untangling the correlations of different traits.

The overall patterns resolved in the multi-predictor model generally reflect the results of Dale et al. (2015) which demonstrates that these variables can be used to predict sexual dichromatism in passerine birds. In addition to this however, it presents some novel patterns on the spatial organisation of colour within both sexes. Examination of the images in Figure 4-2, there is no obvious overall pattern to the spatial organisation. It is evident however, that the spatial organisation of colouration seems to be more pronounced in females than in males. These results also show that in both sexes, species with larger body size seem to have increase elaboration in the bill. Sexual selection affects males and females differently, where males become more elaborately coloured and females become less elaborately coloured the more polygynous a species is. This has been shown previously that males in polygynous mating systems are more elaborately coloured than monogamous species (Moller & Pomiankowski, 1993). In contrast, the females of these species become more dull coloured, especially if males do not partake in parental care, which is often the case in polygynous species (Slagsvold & Lifjeld, 1994).

A novel finding from my study however is the demonstration of elaboration of colour in the bill of females in highly polygynous species. Migration seems to have the least significant correlation with colour, however species that migrate seem to have duller coloured females and brighter coloured males than resident species. Tropical life-history seems to correlate with elaborate colouration and this is a finding that has been suggested before, with species in the tropics often being more elaborately coloured than species in temperate regions (Dale et al., 2015). It is also possible that both sexes in species with tropical life-histories are more colourful in the front of the body, than are species away from tropical living, in more variable environments. Cooperative breeding species also correlate with brightly coloured plumage. Both tropical life-history and cooperative breeding correlate with elaborately coloured irises.

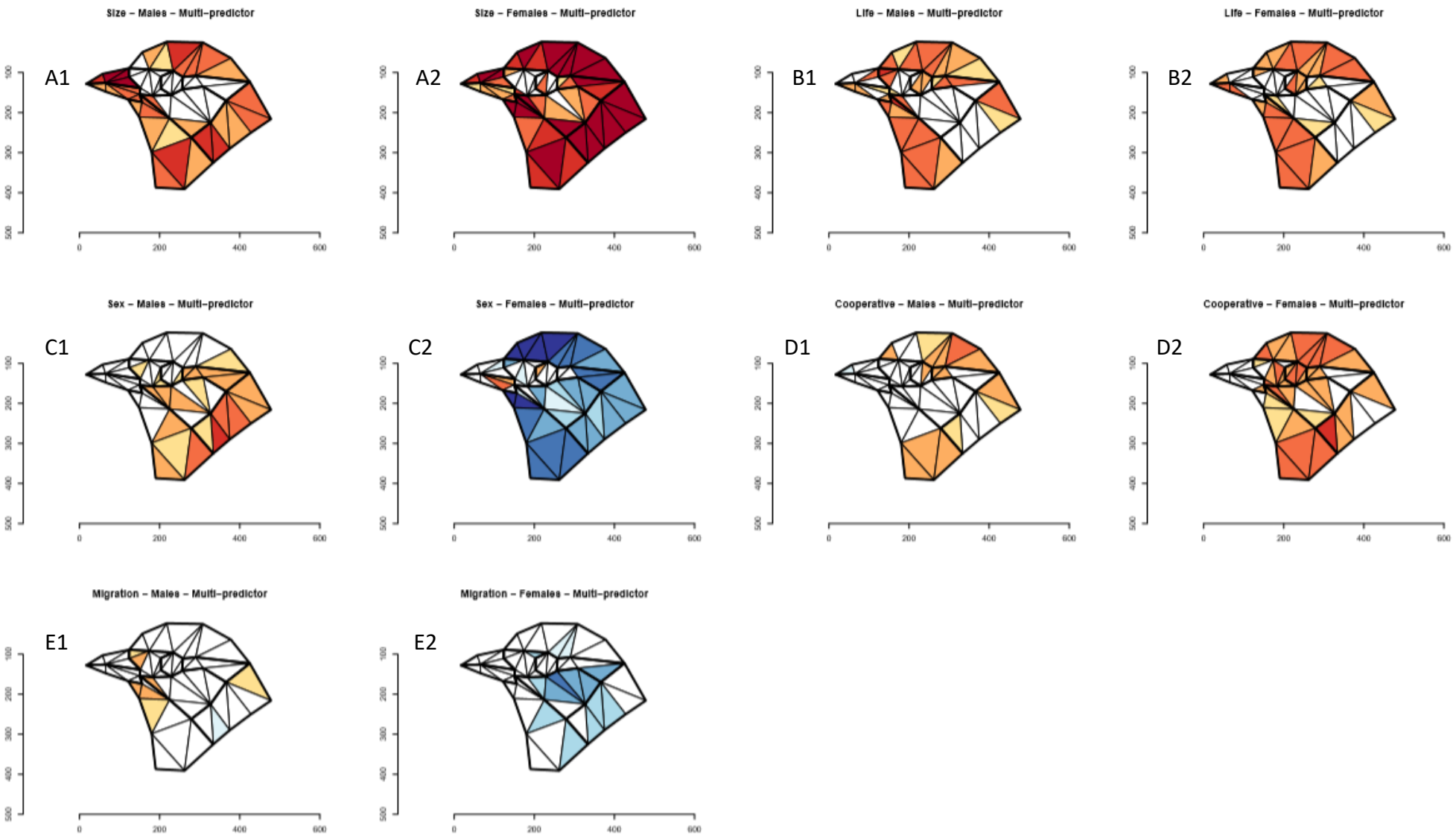

Figure 4-2 Results from the multiple predictor model, using composite traits where A – Body size (body mass and wingspan), B – Tropical life-history (latitude, seasonality, and clutch size), C – Sexual selection (mating system, paternal care, and SSD), D – Cooperative mating, and E – Migration. Where 1 – male and 2 – female. Red indicates a significant positive correlation, blue indicates a significant negative correlation, and white indicates no significant correlation, the darker the colour the more significant the correlation.

4.8 Discussion

This is the first study to utilise a high-resolution spatial approach to test whether evolutionary drivers of elaborate colouration are spatially clumped to certain physical areas on the bodies of birds. This study was successful in looking at correlations between colour elaboration and composite life-history traits in both sexes. Body size, sexual selection, tropical life-history, and cooperative breeding were found to be able to predict colour elaboration in passerine birds. This is not a novel finding however, as this was found described in Dale et al. (2015) and numerous other studies. What is novel about this study is the finding that elaborate bill colour correlates with body size; elaborate iris colour correlates with tropical life-history and cooperative breeding; and the spatial arrangement of colour patterns are more evident and consistent in males than they are in females. Additionally, it seems that females in polygynous mating systems are more likely to have colourful bills than are females with monogamous life histories. Whilst this does not resolve the spatial organisation of colour entirely, it shows that there are certain patterns worth looking into further.

One important new pattern is that bill colour elaboration is positively correlated with body size: the larger the species the more likely it is that the bill is elaborately coloured in both sexes. Larger species of birds have previously been revealed to be more ornamentally coloured than smaller species (Dale et al., 2015). This is thought to be due to larger body size reducing predation risk (Ricklefs, 2010) and therefore potentially removing selection pressures that favour camouflage. Having more elaborated bill colouration in larger species, whose plumage is also more elaborated, could indicate a constraint on bill colour. For example, if plumage is colourful then bill colour could also be colourful as a pleiotropic effect. However, many larger-bodied species of passerines (such as the Eurasian blackbird (*Turdus merula*), the Indian Myna (*Acridotheres tristis*), and Indian ring-necked parrot (*Psittacula krameri*)) have relatively dull coloured or camouflaged plumage with elaborately coloured bills. This suggests instead a function for bill colouration elaboration that may be increasingly important in larger species. For example, it has been suggested that large body size is an evolutionary response to increased competition (Robinson-Wolrath & Owens, 2003). Due to bill colouration signalling dominance (Dey et al., 2015) it is plausible that increased bill colouration in larger species is due to a higher degree of competitive social interactions. This would be an interesting area of research to explore further through looking at correlations between body size and different life-history traits.

Body size also seems to affect colour elaboration in females more so than in males. Indeed this relationship seems to hold true for all variables: the spatial arrangement of colouration seems to affect females more than males. Sexually dichromatic species often evolve due to a change in the plumage colour of females (Badyaev & Hill, 2003a). The allocation of parental care provides an example that highlights this phenomenon. Female birds are often the sex that provide majority of parental care, meaning that they are the sex that spend the most time in the nest. When sitting in the nest, the dorsal area of a bird is often exposed and visible to potential predators (Dunn et al., 2015). Natural selection acts on the visible plumage to be camouflaged to reduce the risk of predation (Martin & Badyaev, 1996). Considering females are often the parent that allocate more energy to parental care (Goymann, Makomba, Urasa, & Schwabl, 2015), this selection for the spatial arrangement in colouration may be more important

in females than in males.

Iris colouration in both sexes of species that have tropical life-histories and are cooperative breeders tend to be more elaborately coloured. The function of eye colouration in communication in birds has long been discussed, but is still largely unknown. However, an experimental study in the European jackdaw (*Corvus monedula)* (Davidson, Clayton, & Thornton, 2014) has demonstrated that iris colour in this species functions to mediate social interactions over nesting cavities. Previous comparative studies (Craig & Hulley, 2004; Davidson, Thornton, & Clayton, 2017) were not able to find correlations between social traits and iris colouration. My study could therefore be the first comparative study to validate this hypothesis using thousands of species and this pattern warrants further investigation in future research.

Because I only analysed the head region of birds, it is possible that there are additional signalling locations on other parts of the body. In Delhey's (2019) study, the wings and tail of birds are more colourful than the back of birds, which indicates an importance in these regions for signalling. These are areas of the body that are used for physical displays. A previous study (Hausmann et al., 2003) demonstrated that areas of plumage that were physically moved or vibrated during mating displays were shown to often correlate with having ultraviolet reflective plumage. This indicates that possibly only areas that are viewed by conspecifics during interactions (like the head and chin) and areas that attention is drawn to using mechanical movement are elaborately coloured to communicate certain information. Performing an analysis for the entire body of birds would help to clear this up.

In conclusion, this study gave some indications of physical areas where colouration is enhanced in birds of different life-histories, which in turn potentially suggests the functions of those areas in signalling (for example the iris in cooperatively breeding species). To be able to resolve these relationships further, more research is necessary. Using the whole body reference template (developed in Chapter 3) could potentially expand on these results and is therefore encouraged. Including more species, as well as potentially other life-history traits, in the analysis of correlations could also help to resolve the relationship between colouration and spatial organisation.

Chapter 5 Overall conclusions and thesis discussion

The research in this thesis investigated avian colouration using two innovative approaches. These approaches build on existing research and provide new considerations of the realm of avian colouration. Whilst the first study on carotenoid colouration was unsuccessful in producing results that support my hypothesis, this study is still valuable in that it provides a method for studying the costs of carotenoid-pigmented signalling in future studies. The second and third study contribute research to an area of avian colouration that is currently undervalued. The spatial organisation of colouration on birds is a topic that is only recently gaining attention. I was able to develop a methodology that allows for the exploration of this relationship, as well as contributing some insight to this field. This thesis demonstrates that conspicuous colouration is the most evident in the front of birds, and also that there is some spatial arrangements to colouration on birds' bodies. Whilst this study does not entirely resolve the spatial patterns of colouration in birds, it provides a framework and a foundation for research in this area to delve deeper.

Whilst my study on the cost of carotenoid pigmented colour elaboration was not successful, the study framework is. Existing research suggests that the cost of carotenoid pigmentation is a social one. The experimental study by Pryke and Andersson (2003) and the comparative study by Dey, Valcu, Kempenaers, and Dale (2015) were able to suggest this as being the case. However to support this hypothesis more research is necessary. I was able to use components from Bright & Waas' (2002) study, as well as from Pryke, Andersson, and Lawes' (2001) study to create an experimental design that considers the potential social cost of carotenoid pigmentation. This leaves a well thought out method for testing this hypothesis in future studies.

Additionally, the research in chapter two also questions certain differences between my study and Bright & Waas' (2002) study. The territorial males in their study responded very aggressively to their intruder models, where I did not find any evidence of aggressive responses in my data. The differences between the studies could potentially help in understanding this: time of the year; location; different population; and the inclusion of the three-dimensional (3D) printed models. Studies that look at the difference in behaviour of birds in response to these variables could potentially be fruitful but will also allow for further research on costs of carotenoid pigmentation. In terms of the next most vital step in this study, I would consider that assessing the use of 3D printed models in behavioural studies as high priority. The use of 3D printed models in behavioural studies has not been previously validated, however it is becoming a common practice to use these models (Anderson et al., 2016).

The study by Delhey (2019) had similar aims to my study by looking at the spatial organisation of colour using a difference in sexual dichromatism. This study was published during the writing of this thesis, and whilst it is very similar, my study was able to resolve spatial organisation to a higher resolution. This is because my analysis looked at patterns of colour elaboration at the pixel level in thousands of species, making it a more statistically powerful method and study. Delhey's (2019) study

included non-passerine species as well as passerines, which is one of the differences between our studies. The method that I developed in chapter three could potentially be used to look at these difference in colour elaboration between passerine and non-passerine species. It could also permit the inclusion of more species, comparing methods of colour quantification, exploring spatial organisation of colour in other taxa, as well as considering questions outside of avian colouration (such as morphometric studies for example).

The results and predictions from chapter four are novel, and whilst no huge overall pattern of colouration was demonstrated from this study (except for iris colouration, bill colouration, and spatial colouration affecting females more than males), it is the beginning of considering bird colouration in this way. Including more species, as well as examining patterns throughout the entire body in chapter four is the next logical step for this research. Beyond that, it is possible to include other life-history traits to see whether there are different areas on the body related to those traits. Traits such as territoriality, sociality, predator prevalence, and nest shape could help to explore the spatial organisation of colouration.

6 References

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7 Appendix 1

Table 1 RGB (red, green, blue) values for the paint used to paint the bills of the models included in the study in Chapter 2. These RGB values are obtained from images of the male blackbirds used in this study (represented by the colour zapper values) and the paint colours were chosen to match these values as closely as possible.

			Paint value			Colour zapper value		
	Colours selected		R	G	B	R	G	B
Bill	Bright male	Rose of Sharon	172	81	45	182.083	63.790	24.383
	Dull male	Aloha	197	128	25	207.596	137.705	30.966
	Juvenile	Woodburn	70	54	41	61.186	50.520	42.563
Feathers	Male feathers	All black	24	24	24			
		Kina brown	67	61	56			
	Juvenile feathers	Woodbark	48	38	33			
		Café royale	106	73	40			

8 Appendix 2

8.1 Script for R (v 3.5.1) code for warping the images of the heads of birds

```
library(imager)
library(sp)
data_file_path = "C:\\Users\\....image_polygon_coordinates.csv"
data = read.csv(data_file_path)
setwd(dirname(data_file_path))
source("Warp_Functions.R")
DIRfiles<-"C:\\Users\\....\\passerine_originals\\select_me.txt"
setwd(dirname(DIRfiles))
observer = "name"
#Register the image
data = read.csv(data_file_path) #refresh data set so last bird is updated
data$scorer = as.character(data$scorer)
data$comment = as.character(data$comment)
working_list = subset(data, dryad == 1 & registered == 0)
print(c(nrow(working_list),"images left to go"))
working_list = list(as.numeric(rownames(working_list)))
working_list = working_list[[1]][sample(1:length(working_list[[1]]))]
i = 1  #this will ultimately go in a for loop
dev.new()
focal = working_list[[1]][i]
bird <- load.image(as.character(data$image_path[focal])) #chooses non-chosen images
plot(bird, main =as.character(data$image_id[focal])   )
source("image_registration_for_heads.r")
canvas = load.image("blank.jpg")
arr = as.array(canvas)
focal_polygons = list(ub, lb, lr, ch, au, cr, es, ey, np)
for(i in 1:length(polygons)) {
        arr = remap_function(matrixes[[i]], polygons[[i]], focal_polygons[[i]], arr, bird)
        canvas = as.cimg(arr)
        plot(canvas, main =c(as.character(data$image_id[focal]),"warped")  )
        for(p in 1:i) {
                polygon(polygons[[p]], border = colours[[p]], lwd = 3)}}
print("comment about this image?")
data$comment[focal] = readline("") #run the code to here
#then run the code below if you are happy with it, otherwise start from register the image
above
#save the coordinates and image if you are happy with it
data$scorer[focal] = observer
data$registered[focal] = 1
unfiled_path =
gsub("[\\\\]|[^[:print:]]","/",paste(gsub("select_me.txt","",gsub("passerine_originals","pass
erine_warped_images",DIRfiles)),"unfiled\\",data$image_id[focal],".warped",".jpg",sep =
""))
save.image(canvas,unfiled_path)
write.csv(data,data_file_path, row.names = FALSE)
```

8.2 Script for R (v 3.5.1) code for warping images of the entire body of birds

```
library(imager)
library(sp)
#make sure remap_function is loaded
remap_function = function(matrix, polygon, tmp, array, bird) {
	#Create Array
	bird_array = as.array(bird)
	#For each triangle in polygon
	for (i in 1:(length(matrix)/3)) {
		temp = c(matrix[1+(i-1)*3],matrix[2+(i-1)*3],matrix[3+(i-1)*3])
		r1 = as.list(as.data.frame(polygon)[temp,])
		p1 = as.list(as.data.frame(tmp)[temp,])
		fit = lm(cbind(p1$x ,p1$y) ~ r1$x + r1$y)
		a = t(coef(fit))
		# Select corners of triangle
		v1 = c(r1$x[1], r1$y[1])
		v2 = c(r1$x[2], r1$y[2])
		v3 = c(r1$x[3], r1$y[3])
		# Iterate through rectangle of pixels
		for (y in (min(r1$y)-1):(max(r1$y)+1)) {
			for (x in (min(r1$x)-1):(max(r1$x)+1)) {
				# Pixel Point
				p1 = c(x,y)
				# Calculate Barycentric Coordinates
				b1 = ((p1[1] - v2[1]) * (v1[2] - v2[2]) - (v1[1] - v2[1]) * (p1[2] -
v2[2])) < 0
				b2 = ((p1[1] - v3[1]) * (v2[2] - v3[2]) - (v2[1] - v3[1]) * (p1[2] -
v3[2])) < 0
				b3 = ((p1[1] - v1[1]) * (v3[2] - v1[2]) - (v3[1] - v1[1]) * (p1[2] -
v1[2])) < 0
				if(((b1 == b2) && (b2 == b3))) {
					nx = a[1,1] + x*a[1,2] + y*a[1,3]
					ny = a[2,1] + x*a[2,2] + y*a[2,3]
					nx = round(nx,0)
					ny = round(ny,0)
					array[x,y,,] = bird_array[nx,ny,,]}}}}
	return(array)}
#Define triangular mesh for each polygon
#HEAD
upperBillmatrix = c(1,2,6,2,3,6,3,4,6,4,5,6)
lowerBillmatrix = c(1,2,7,2,3,7,3,5,7,5,6,7,3,4,5)
lorematrix = c(1,2,10,2,9,10,2,7,9,7,8,9,2,4,7,2,3,4,4,5,7,5,6,7)
chinmatrix = c(8,9,10,2,8,10,1,2,10,2,7,8,2,3,7,3,5,7,3,4,5,5,6,7)
auriclematrix = c(7,8,9,9,1,7,1,5,7,2,3,5,1,2,5,3,4,5,5,6,7)
crownmatrix = c(1,2,10,2,3,10,3,9,10,3,4,9,4,8,9,4,7,8,4,5,7,5,6,7)
eyestripematrix = c(1,2,6,2,5,6,2,5,3,3,4,5)
eyematrix = c(1,5,6,1,4,5,1,2,4,2,3,4)
```

```
napematrix = c(1,2,8,8,2,3,8,3,4,7,8,4,7,4,5,5,6,7)
#BODY
mantlematrix = c(4,5,6,3,4,6,3,6,10,6,7,10,7,8,10,8,9,10,1,2,10,2,3,10)
m_covertsmatrix = c(1,2,3,1,3,5,3,4,5,1,5,6)
g_covertsmatrix = c(4,5,6,3,4,6,2,3,6,2,6,7,1,2,7,1,7,8)
secondariesmatrix = c(1,2,3,1,7,6,1,6,3,3,6,4,4,5,6)
primariesmatrix = c(4,5,6,4,6,7,3,4,7,3,7,8,1,3,8,1,2,3)
rumpmatrix = c(1,8,9,1,2,8,2,7,8,2,3,7,3,6,7,3,5,6,3,4,5)
tailmatrix = c(1,2,9,2,3,4,2,4,9,4,5,9,5,6,7,5,7,9,7,8,9)
ventmatrix = c(1,2,4,2,3,4)
bellymatrix = c(1,2,8,1,8,9,2,3,4,2,4,5,2,5,8,5,6,7,5,7,8)
l_breastmatrix = c(2,3,4,2,4,5,1,2,5,1,5,7,5,6,7)
u_breastmatrix = c(1,2,3,3,4,5,3,5,6,1,3,6,1,6,8,6,7,8)
legmatrix =
c(1,14,15,1,2,12,1,12,13,1,13,14,2,11,12,2,8,11,2,7,8,2,3,7,3,6,7,3,5,6,3,4,5,8,9,10,8,10,11)
#locate the master data files
#DATA FOR HEADS
data_file_path =   "C:\\Users\\...\\image_polygon_coordinates.csv"
data = read.csv(data_file_path)
#DATA FOR BODY
data2_file_path = "C:\\Users\\...\\image_polygon_coordinates_BODY.csv"
data2 = read.csv(data2_file_path)
observer = "name"
#Reference Bird
#HEAD (based on average of tanagers)
upperBill = list(x=as.numeric(data[1,16:21]), y=as.numeric(data[1,22:27]))
lowerBill = list(x=as.numeric(data[1,28:35]), y=as.numeric(data[1,36:43]))
lore = list(x=as.numeric(data[1,44:53]), y=as.numeric(data[1,54:63]))
chin = list(x=as.numeric(data[1,64:73]), y=as.numeric(data[1,74:83]))
auricle  = list(x=as.numeric(data[1,84:92]), y=as.numeric(data[1,93:101]))
crown = list(x=as.numeric(data[1,102:111]), y=as.numeric(data[1,112:121]))
eyestripe = list(x=as.numeric(data[1,122:127]), y=as.numeric(data[1,128:133]))
eye = list(x=as.numeric(data[1,134:139]), y=as.numeric(data[1,140:145]))
nape = list(x=as.numeric(data[1,146:153]), y=as.numeric(data[1,154:161]))
#BODY - based on bird 197.128.0
mantle = list(x=as.numeric(data2[1,16:25]), y=as.numeric(data2[1,26:35]))
m_coverts = list(x=as.numeric(data2[1,36:41]), y=as.numeric(data2[1,42:47]))
g_coverts = list(x=as.numeric(data2[1,48:55]), y=as.numeric(data2[1,56:63]))
secondaries = list(x=as.numeric(data2[1,64:70]), y=as.numeric(data2[1,71:77]))
primaries = list(x=as.numeric(data2[1,78:85]), y=as.numeric(data2[1,86:93]))
rump = list(x=as.numeric(data2[1,94:102]), y=as.numeric(data2[1,103:111]))
tail = list(x=as.numeric(data2[1,112:120]), y=as.numeric(data2[1,121:129]))
vent = list(x=as.numeric(data2[1,130:133]), y=as.numeric(data2[1,134:137]))
belly = list(x=as.numeric(data2[1,138:146]), y=as.numeric(data2[1,147:155]))
l_breast = list(x=as.numeric(data2[1,156:162]), y=as.numeric(data2[1,163:169]))
u_breast = list(x=as.numeric(data2[1,170:177]), y=as.numeric(data2[1,178:185]))
leg = list(x=as.numeric(data2[1,186:200]), y=as.numeric(data2[1,201:215]))
```

```
blank = load.image("C:\\Users\\....\\tanager_images\\full_body_blank.jpg")
plot(blank)
#reference polygons
polygons = list(upperBill, lowerBill, lore, chin, auricle, crown, eyestripe, eye, nape, mantle,
m_coverts, g_coverts, secondaries, primaries, rump, tail, vent, belly, l_breast, u_breast, leg)
colours = list("blue", "purple", "red", "green", "yellow", "orange", "coral2", "mediumorchid",
"springgreen3", "dodgerblue", "yellowgreen", "hotpink", "darkviolet", "limegreen", "tan4",
"darkslateblue", "firebrick3", "lawngreen", "royalblue2", "magenta3", "black")
mesh_list = list( upperBillmatrix, lowerBillmatrix, lorematrix, chinmatrix, auriclematrix,
crownmatrix, eyestripematrix, eyematrix, napematrix, mantlematrix, m_covertsmatrix,
g_covertsmatrix, secondariesmatrix, primariesmatrix, rumpmatrix, tailmatrix, ventmatrix,
bellymatrix, l_breastmatrix, u_breastmatrix, legmatrix)
#draw polygons
for(i in 1:length(polygons)) {
        polygon(polygons[[i]], border = colours[[i]], lwd = 3)}
#draw mesh
for(j in 1:length(mesh_list))
{focal_matrix = mesh_list[j]
focal_colour = col2rgb(colours[j])
for (i in 1:(length(focal_matrix[[1]])/3))
{temp = c(focal_matrix[[1]][1+(i-1)*3],focal_matrix[[1]][2+(i-1)*3],focal_matrix[[1]][3+(i-
1)*3])
temp = as.list(as.data.frame(polygons[[j]])[temp,])
polygon(temp,col=rgb(focal_colour[1,1]/255, focal_colour[2,1]/255,
focal_colour[3,1]/255,0.5))}}
#draw a branch #branch = locator(2, type = "p", col = "orange")
segments(470,732,434,870, lwd = 2)
segments(406,982,401,999, lwd = 2)
segments(503,959,493,999, lwd = 2)
segments(509,924,527,850, lwd = 2)
segments(549,762,537,805, lwd = 2)
# start here for each new bird
DIRfiles <- "C:\\Users\\...\\passerine_originals\\select_me.txt"
DIRfiles2 <- "C:\\Users\\...\\HBW birds - passerines\\select_me.txt"
setwd(dirname(DIRfiles))
data2 = read.csv(data2_file_path) # refresh data set so last bird is updated
data2$scorer = as.character(data2$scorer)
data2$comment = as.character(data2$comment)
working_list = subset(data2, dryad == 1)
halfdone = working_list[c("jim_pk","registered")]
halfdone$jim_pk = as.factor(halfdone$jim_pk)
halfdone = subset(halfdone, registered == 1)
halfdone$jim_pk = as.factor(as.character(halfdone$jim_pk))
halfdone2 = as.data.frame(table(halfdone))
halfdone2 = subset(halfdone2, Freq  == 1)
templist = list(as.numeric(as.character(halfdone2$jim_pk)))
data2$jim_pk %in% templist[[1]]
```

```
working_list = subset(data2, registered == 0 & jim_pk %in% templist[[1]])
nrow(working_list)
print(c(nrow(working_list),"images left to go"))
working_list = list(as.numeric(rownames(working_list)))
working_list = working_list[[1]][sample(1:length(working_list[[1]]))]
i = 1
focal = working_list[[1]][i]
#set up head shot to get key points
setwd(dirname(DIRfiles))
image_path= as.character(data2$image_path[focal])
bird = load.image(image_path)
plot(bird)
ub = list(x=as.numeric(data[focal,16:21]), y=as.numeric(data[focal,22:27]))
lb = list(x=as.numeric(data[focal,28:34]), y=as.numeric(data[focal,36:42]))# note not using
that last duplicated point
lr = list(x=as.numeric(data[focal,44:53]), y=as.numeric(data[focal,54:63]))
ch = list(x=as.numeric(data[focal,64:73]), y=as.numeric(data[focal,74:83]))
au  = list(x=as.numeric(data[focal,84:92]), y=as.numeric(data[focal,93:101]))
cr = list(x=as.numeric(data[focal,102:111]), y=as.numeric(data[focal,112:121]))
es = list(x=as.numeric(data[focal,122:127]), y=as.numeric(data[focal,128:133]))
ey = list(x=as.numeric(data[focal,134:139]), y=as.numeric(data[focal,140:145]))
np = list(x=as.numeric(data[focal,146:153]), y=as.numeric(data[focal,154:161]))
polygon(ub, border = "blue", lwd = 3)
polygon(lb, border = "purple", lwd = 3)
polygon(lr, border = "red", lwd = 3)
polygon(ch, border = "green", lwd = 3)
polygon(au, border = "yellow", lwd = 3)
polygon(cr, border = "blue", lwd = 3)
polygon(es , border = "purple", lwd = 3)
polygon(ey, border = "black", lwd = 3)
polygon(np , border = "red", lwd = 3)
points(np$x[2:5],np$y[2:5], pch = 21, col = "red", bg = "white")
points(ch$x[5:6],ch$y[5:6], pch = 21, col = "red", bg = "white")
#Plot full bird
setwd(dirname(DIRfiles2))
full_bird = load.image(image_path)
dev.new()
plot(full_bird)
#Mantle
print("go for mantle - 10 of 10 points")
flush.console()
ma = locator(10, type = "p", col = "orange")
ma$x = round(ma$x,0)
ma$y = round(ma$y,0)
polygon(ma, border = "blue", lwd = 3)
points(ma, pch = 21, col = "red", bg = "white")
data2[focal,16:25] = ma$x
```

```
data2[focal,26:35] = ma$y
data2[focal,16:25]
data2[focal,26:35]
#Median coverts
print("go for median coverts -3 of 6 points")
flush.console()
mc = locator(3, type = "p", col = "orange")
mc$x = round(mc$x,0)
mc$y = round(mc$y,0)
mc$x[4] = ma$x[5]
mc$y[4] = ma$y[5]
mc$x[5] = ma$x[6]
mc$y[5] = ma$y[6]
mc$x[6] = ma$x[7]
mc$y[6] = ma$y[7]
polygon(mc, border = "purple", lwd = 3)
points(mc, pch = 21, col = "red", bg = "white")
data2[focal,36:41] = mc$x
data2[focal,42:47] = mc$y
data2[focal,36:41]
data2[focal,42:47]
#Greater coverts
print("go for greater coverts - 4 of 8 points")
flush.console()
gc = locator(4, type = "p", col = "orange")
gc$x = round(gc$x,0)
gc$y = round(gc$y,0)
gc$x[5] = mc$x[2]
gc$y[5] = mc$y[2]
gc$x[6] = mc$x[1]
gc$y[6] = mc$y[1]
gc$x[7] = mc$x[6]
gc$y[7] = mc$y[6]
gc$x[8] = ma$x[8]
gc$y[8] = ma$y[8]
polygon(gc, border = "red", lwd = 3)
points(gc,  pch = 21, col = "red", bg = "white")
data2[focal,48:55] = gc$x
data2[focal,56:63] = gc$y
data2[focal,48:55]
data2[focal,56:63]
#Secondaries
print("go for secondaries - 4 of 7 points")
flush.console()
se = locator(4, type = "p", col = "orange")
se$x = round(se$x,0)
se$y = round(se$y,0)
```

```
se$x[5] = gc$x[8]
se$y[5] = gc$y[8]
se$x[6] = gc$x[1]
se$y[6] = gc$y[1]
se$x[7] = gc$x[2]
se$y[7] = gc$y[2]
polygon(se, border = "yellow", lwd = 3)
points(se, pch = 21, col = "red", bg = "white")
data2[focal,64:70] = se$x
data2[focal,71:77] = se$y
data2[focal,64:70]
data2[focal,71:77]
#Primaries
print("go for primaries - 4 of 8 points")
flush.console()
pr = locator(4, type = "p", col = "orange")
pr$x = round(pr$x,0)
pr$y = round(pr$y,0)
pr$x[5] = gc$x[3]
pr$y[5] = gc$y[3]
pr$x[6] = gc$x[2]
pr$y[6] = gc$y[2]
pr$x[7] = se$x[1]
pr$y[7] = se$y[1]
pr$x[8] = se$x[2]
pr$y[8] = se$y[2]
polygon(pr, border = "blue", lwd = 3)
points(pr, pch = 21, col = "red", bg = "white")
data2[focal,78:85] = pr$x
data2[focal,86:93] = pr$y
data2[focal,78:85]
data2[focal,86:93]
#Rump
#Note Rump is a special polygon that is drawn completely but then assigned completely to
previously defined coordinates
print("go for rump - 9 of 9 points")
flush.console()
ru = locator(9, type = "p", col = "orange")
ru$x = round(ru$x,0)
ru$y = round(ru$y,0)
polygon(ru, border = "orange", lwd = 3)
points(ru, pch = 21, col = "red", bg = "white")
data2[focal,94:102] = ru$x
data2[focal,103:111] = ru$y
data2[focal,94:102]
data2[focal,103:111]
#Tail (special polygon)
```

```
print("go for tail - 9 of 9 points")
flush.console()
tl = locator(9, type = "p", col = "orange")
tl$x = round(tl$x,0)
tl$y = round(tl$y,0)
polygon(tl, border = "red", lwd = 3)
points(tl, points(ru, pch = 21, col = "red", bg = "white")
data2[focal,112:120] = tl$x
data2[focal,121:129] = tl$y
data2[focal,112:120]
data2[focal,121:129]
#Vent (special polygon)
print("go for VENT - 4 OF 4 points")
flush.console()
ve = locator(4, type = "p", col = "orange")
ve$x = round(ve$x,0)
ve$y = round(ve$y,0)
#point 2 needs to be part of the belly
polygon(ve, border = "yellow", lwd = 3)
points(ve,  pch = 21, col = "red", bg = "white")
data2[focal,130:133] = ve$x
data2[focal,134:137] = ve$y
data2[focal,130:133]
data2[focal,134:137]
#Belly(includes shanks)
print("go for belly - 6 of 9 points")
flush.console()
be = locator(6, type = "p", col = "orange")
be$x = round(be$x,0)
be$y = round(be$y,0)
be$x[7] = pr$x[5]
be$y[7] = pr$y[5]
be$x[8] = pr$x[4]
be$y[8] = pr$y[4]
be$x[9] = ve$x[2]
be$y[9] = ve$y[2]
polygon(be, border = "purple", lwd = 3)
points(be, pch = 21, col = "red", bg = "white")
data2[focal,138:146] = be$x
data2[focal,147:155] = be$y
data2[focal,138:146]
data2[focal,147:155]
#Lower breast
print("go for LOWER BREAST  - 3 OF 7 points")
flush.console()
bl = locator(3, type = "p", col = "orange")
bl$x = round(bl$x,0)
```

```
bl$y = round(bl$y,0)
bl$x[4] = gc$x[5]
bl$y[4] = gc$y[5]
bl$x[5] = gc$x[4]
bl$y[5] = gc$y[4]
bl$x[6] = gc$x[3]
bl$y[6] = gc$y[3]
bl$x[7] = be$x[6]
bl$y[7] = be$y[6]
polygon(bl, border = "light blue", lwd = 3)
points(bl,  pch = 21, col = "red", bg = "white")
data2[focal,156:162] = bl$x
data2[focal,163:169] = bl$y
data2[focal,156:162]
data2[focal,163:169]
#Upper breast
print("go for UPPER BREAST  - 3 OF 8 points")
flush.console()
bu = locator(3, type = "p", col = "orange")
bu$x = round(bu$x,0)
bu$y = round(bu$y,0)
bu$x[4] = ma$x[4]
bu$y[4] = ma$y[4]
bu$x[5] = ma$x[5]
bu$y[5] = ma$y[5]
bu$x[6] = mc$x[3]
bu$y[6] = mc$y[3]
bu$x[7] = mc$x[2]
bu$y[7] = mc$y[2]
bu$x[8] = bl$x[3]
bu$y[8] = bl$y[3]
polygon(bu, border = "blue", lwd = 3)
points(bu,pch = 21, col = "red", bg = "white")
data2[focal,170:177] = bu$x
data2[focal,178:185] = bu$y
data2[focal,170:177]
data2[focal,178:185]
#Leg
print("go for LEG  - 13 of 15 points")
flush.console()
lg = locator(13, type = "p", col = "orange")
lg$x = round(lg$x,0)
lg$y = round(lg$y,0)
lg$x[14] = be$x[4]
lg$y[14] = be$y[4]
lg$x[15] = be$x[3]
lg$y[15] = be$y[3]
```

```
polygon(lg, border = "brown", lwd = 3)
points(lg, pch = 19, col = "blue")
data2[focal,186:200] = lg$x
data2[focal,201:215] = lg$y
data2[focal,186:200]
data2[focal,201:215]
#Two images need to be warped into one as heads were warped separately from the body
canvas = load.image("C:\\Users\\...\\tanager_images\\full_body_blank.jpg")
# Initialise Array
arr = as.array(canvas)
#For head
#Reference polygons
polygons = list(upperBill, lowerBill, lore, chin, auricle, crown, eyestripe, eye, nape)
#Focal Polygons
focal_polygons = list(ub, lb, lr, ch, au, cr, es, ey, np)
#Matrixes
matrixes = list(upperBillmatrix, lowerBillmatrix, lorematrix, chinmatrix, auriclematrix,
crownmatrix, eyestripematrix, eyematrix, napematrix)
#For each polygon
for(i in 1:length(polygons)) {
        # Remap Polygon
        arr = remap_function(matrixes[[i]], polygons[[i]], focal_polygons[[i]], arr, bird)
        #Convert array to canvas
        canvas = as.cimg(arr)
        #Plot canvas
        plot(canvas)
        #Plot Polygons
        for(p in 1:i) {polygon(polygons[[p]], border = colours[[p]], lwd = 3)}}
#For body using different image
#Reference polygons
polygons = list(mantle, m_coverts, g_coverts, secondaries, primaries, rump, tail, vent, belly,
l_breast, u_breast, leg)
#Focal Polygons
focal_polygons = list(ma, mc, gc, se, pr, ru, tl, ve, be, bl, bu, lg)
#Matrixes
matrixes = list( mantlematrix, m_covertsmatrix, g_covertsmatrix, secondariesmatrix,
primariesmatrix, rumpmatrix, tailmatrix, ventmatrix, bellymatrix, l_breastmatrix,
u_breastmatrix, legmatrix)
#For each polygon
for(i in 1:length(polygons)) {
        #Remap Polygon
        arr = remap_function(matrixes[[i]], polygons[[i]], focal_polygons[[i]], arr, full_bird)
        #Convert array to canvas
        canvas = as.cimg(arr)
        #Plot canvas
        plot(canvas)  ###wathc out for dot artifacts
#run to here, if image is ok then:
```

```
data2$scorer[focal] = observer
data2$registered[focal] = 1
write.csv(data2,data2_file_path, row.names = FALSE)  # this causes a crash if scoring is stopped mid-bird
path = paste(dirname(DIRfiles2),"/warped/",as.character(data2$image_id[focal]),".jpg", sep = "")
save.image(canvas,path,quality = 1.0)
dev.off()
dev.off()
```

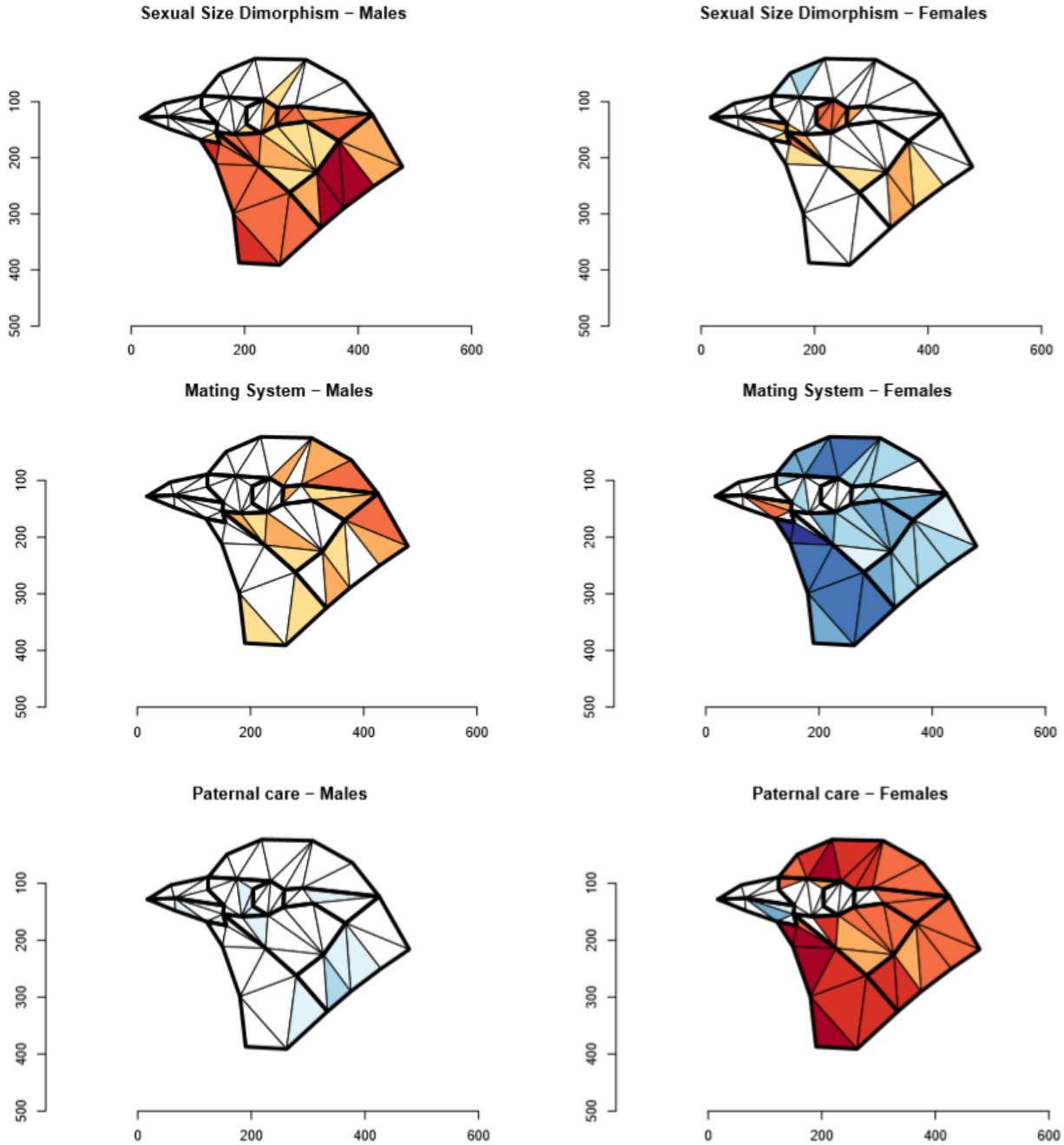
8.3 Script for R (v 3.5.1) code for sexual dichromatism heat map

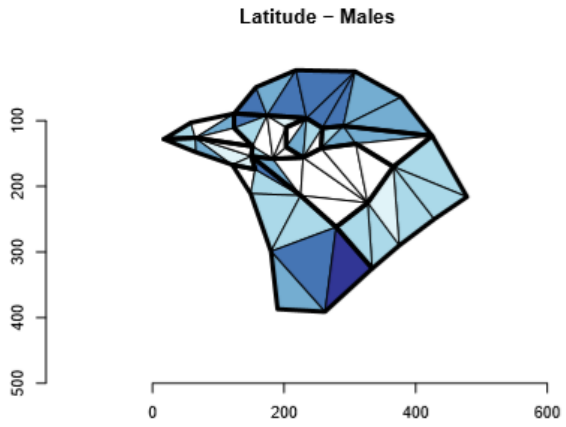
```
library(imager)
library(sp)
#locate the master data file
data_file_path = "C:\\Users\\.....image_polygon_coordinates_BODY.csv"
data = read.csv(data_file_path)
#locate the folder with images
#"now choose the file 'select_me.txt' from the images folder you are working on")
DIRfiles<-"C:\\Users\\....HBW birds - passerines\\warped\\select_me.txt"
setwd(dirname(DIRfiles))
#this code is using all species but can be changed to only include tanager species
dryad = subset(data, dryad == 1 & registered == 1)
nrow(dryad)/2
dryad$jim_pk = as.factor(dryad$jim_pk)
tot = nlevels(dryad$jim_pk)
species_list = levels(dryad$jim_pk)
species_list
DIR.warped.files =  "C:\\Users\\....HBW birds - passerines\\warped\\"
for (i in 1:length(species_list))  # could use length(species_list) instead of tot
#i = 1  ###### this will ultimately go in a for loop (or a function)
{temp = subset(dryad, jim_pk == species_list[i] & sex == 1)
#male <- load.image(as.character(temp$image_path[1])) # original image
male <- load.image(paste(DIR.warped.files,as.character(temp$image_id[1]),".jpg",sep=""))
##bodies are not names .warp.jpg like the heads are, had to change
#plot(male)
temp = subset(dryad, jim_pk == species_list[i] & sex == 0)
female <- load.image(paste(DIR.warped.files,as.character(temp$image_id[1]),".jpg",sep=""))
#plot(female)
distance = (male - female)^2
distance = distance[,,,1] + distance[,,,2] + distance[,,,3]
distance = sqrt(distance)
distance = as.cimg(distance)
#plot(distance)
path = paste(DIR.warped.files,"sex_dim\\",temp$image_id[1],".sexdim.jpg",sep = "")
path = gsub("[\\\\]|[^[:print:]]","/",path)
save.image(distance,path,quality = 1.0) }
#use R 64 bit version for this
l <- load.dir( "F:\\sex_dim")
```

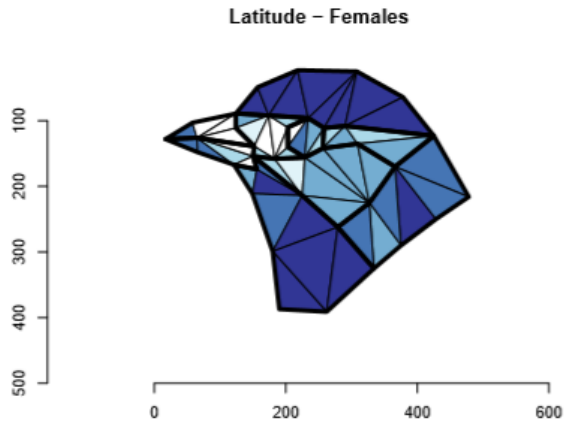
```
l
x = average(l)
#extracting species that have different male and female plates
#locate the master data file
data_file_path =  "C:\\Users\\....\\image_polygon_coordinates_BODY.csv"
data = read.csv(data_file_path)
#find data file for illustration plate attributes
birds = read.csv("C:\\Users\\....\\image_info_HBW.csv")
birds = birds[c("jim_pk", "same_plates" , "sexes_alike", "sameColor_diffImage")]
dirname = "C:\\Users\\.....\\warped\\sex_dim”
#this next code creates an image list of selected images from within a folder
setwd(dirname)
im1 = load.image(paste(as.character(tanagers$image_id[which(tanagers$jim_pk ==
species_list[1] & tanagers$sex == 0)]),".sexdim.jpg",sep=""))
l <- imlist(im1)
#plot(im1)
for (i in 2:n)
{temp = load.image(paste(as.character(tanagers$image_id[which(tanagers$jim_pk ==
species_list[i] & tanagers$sex == 0)]),".sexdim.jpg",sep=""))
m = imlist(temp)
l = as.imlist(c(l,m))}
l
x = average(l)
dirname = "F:\\sex_dim" # change this accordingly
setwd(dirname)
im1 = load.image(paste(as.character(dryad$image_id[which(dryad$jim_pk == species_list[1]
& dryad$sex == 0)]),".sexdim.jpg",sep=""))
l <- imlist(im1)
plot(im1)
for (i in 2:n)
{temp = load.image(paste(as.character(dryad$image_id[which(dryad$jim_pk ==
species_list[i] & dryad$sex == 0)]),".sexdim.jpg",sep=""))
m = imlist(temp)
l = as.imlist(c(l,m))}
l
x = average(l)
p = as.matrix(mirror(imrotate(x,180),"x"))
image(p,col = terrain.colors(15))
contour(p, add = TRUE)
legend(0.8,0.8, legend)
```

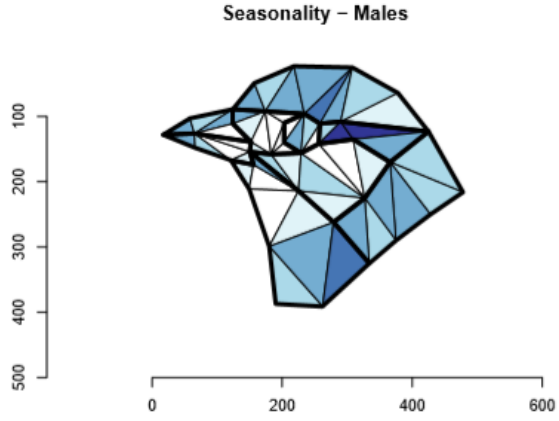
9 Appendix 3

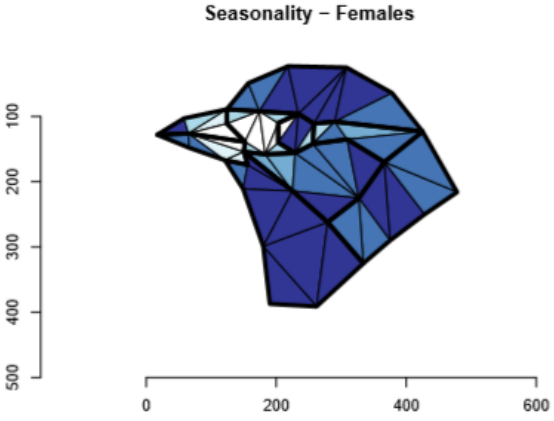
Figure 1 Results from single predictor models where red indicates a significant positive correlation, blue indicates a significant negative correlation, and white indicates no significant correlation, darker colours indicate larger effect sizes.

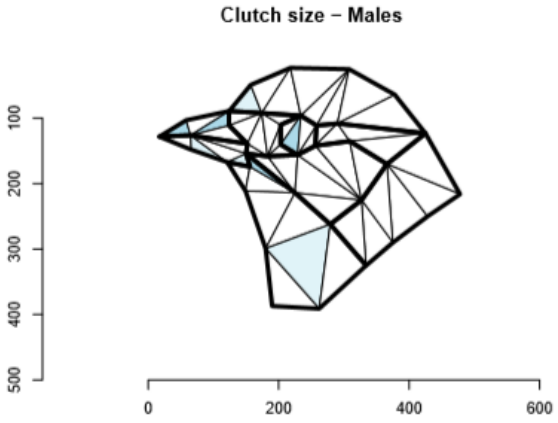


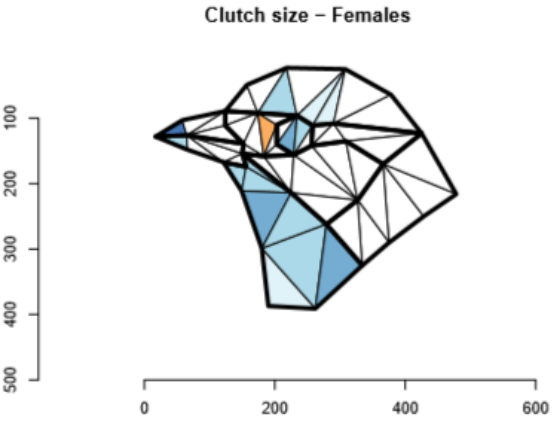

Latitude – Males
100
200
300
400
500
0
200
400
600


Latitude – Females
100
200
300
400
500
0
200
400
600


Seasonality – Males
100
200
300
400
500
0
200
400
600


Seasonality – Females
100
200
300
400
500
0
200
400
600


Clutch size – Males
100
200
300
400
500
0
200
400
600


Clutch size – Females
100
200
300
400
500
0
200
400
600

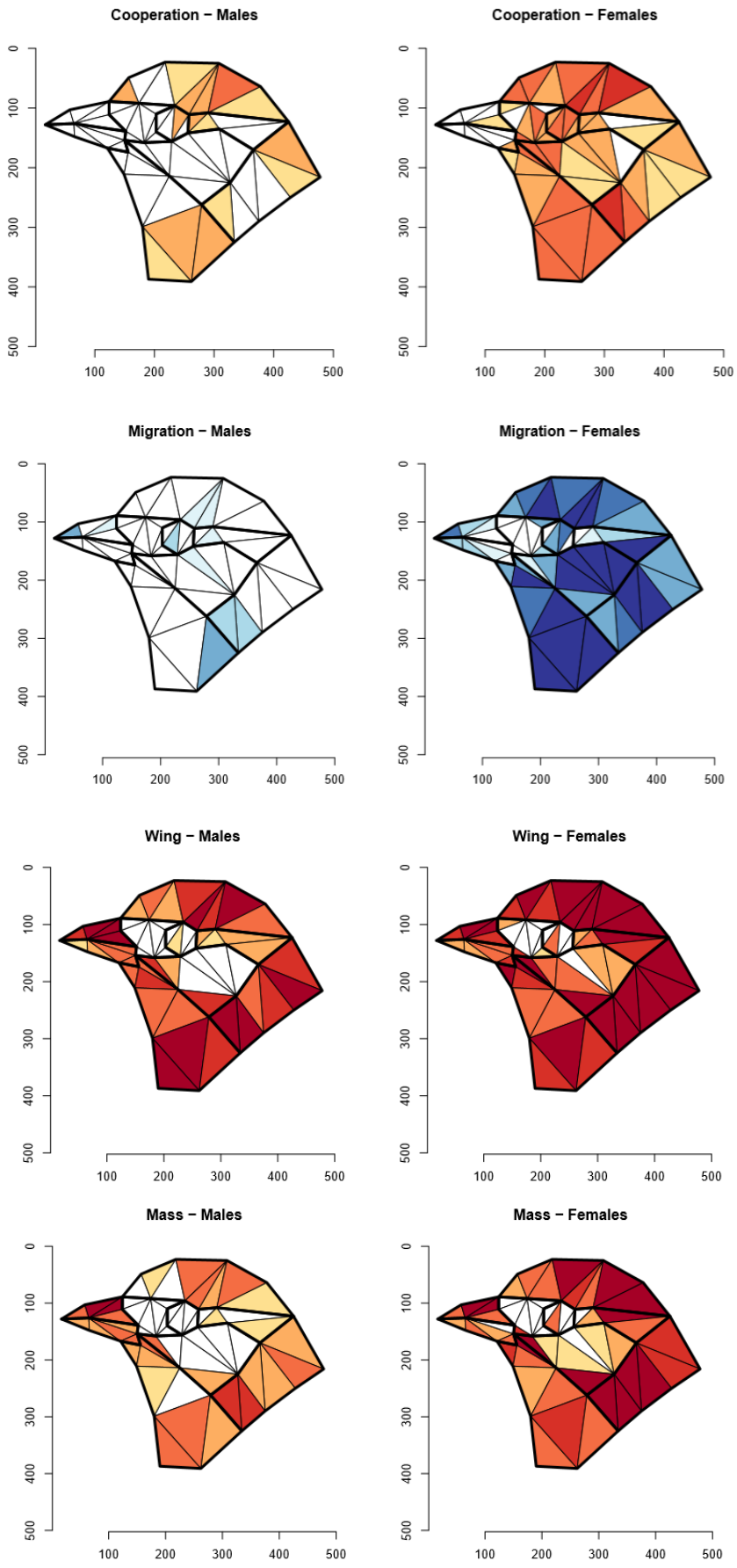

Cooperation – Males
Cooperation – Females
Migration – Males
Migration – Females
Wing – Males
Wing – Females
Mass – Males
Mass – Females
0
100
200
300
400
500

Table 2 Effect sizes and p-values for the correlations between body regions and different life-history traits from the multiple predictor model for each triangle in females.

patch	patch_tri_	f.size.effe	f.size.P	f.life.effe	f.life.P	f.sex.effe	f.sex.P	f.coop.eff	f.coop.P	f.mig.effe	f.mig.P
upperBill	1	0.017149	0.000692	0.009761	0.014486	-0.00346	0.356601	0.000768	0.779475	-0.00598	0.104254
upperBill	2	0.019667	2.25E-05	0.001137	0.761514	-0.00114	0.744346	-6.93E-06	0.997852	-0.0051	0.141135
upperBill	3	0.024759	1.24E-09	0.00139	0.693083	-0.00562	0.079458	0.001646	0.497577	-0.0032	0.330407
upperBill	4	0.013902	0.001689	0.000452	0.89986	-0.00011	0.973346	0.003801	0.12335	-0.00358	0.280354
lowerBill	1	0.009434	0.053054	0.010768	0.005749	0.003089	0.398046	0.003725	0.164139	-0.00123	0.732271
lowerBill	2	0.011185	0.014188	0.010273	0.007164	0.002056	0.559721	0.001721	0.512263	-0.00045	0.898973
lowerBill	3	0.010419	0.046744	0.003739	0.37959	0.012189	0.002167	0.002751	0.346845	-0.00413	0.294043
lowerBill	4	0.009275	0.069386	-0.00073	0.863323	0.009526	0.015553	-4.66E-05	0.987279	0.000481	0.903044
lowerBill	5	0.014576	0.002065	0.005245	0.178501	0.002867	0.428376	0.006275	0.019267	-0.00083	0.818254
lore	1	0.013524	0.03511	0.01143	0.034158	-0.01307	0.008601	0.006162	0.096859	0.001935	0.699231
lore	2	0.004672	0.46457	0.006824	0.195741	0.003145	0.520451	0.003464	0.339345	0.003189	0.514035
lore	3	0.004801	0.450563	-0.00188	0.723419	-0.00929	0.058683	0.007227	0.048268	-0.00057	0.908038
lore	4	0.022256	0.000229	0.00712	0.162158	-0.00405	0.388054	0.01012	0.003903	-4.71E-05	0.992066
lore	5	-0.00017	0.978942	-0.0015	0.779611	-0.00305	0.540337	0.01021	0.005748	0.001666	0.738147
lore	6	0.003949	0.577523	0.0011	0.853426	-0.00605	0.270823	0.002394	0.558787	-0.0129	0.019666
lore	7	-0.00028	0.964732	0.00084	0.879041	0.001716	0.733216	0.00915	0.016213	-0.00316	0.539344
lore	8	0.009615	0.117564	0.004276	0.418928	-0.00303	0.530039	0.008494	0.019809	-0.00559	0.256505
chin	1	0.025573	1.08E-06	0.011091	0.012792	-0.02154	1.44E-07	0.004339	0.156978	-0.00218	0.599002
chin	2	0.027374	7.34E-05	0.017175	0.00293	-0.02479	3.41E-06	0.007321	0.06507	-0.00671	0.210109
chin	3	0.029542	1.48E-05	0.014662	0.012825	-0.00923	0.08555	0.01	0.013768	-0.00418	0.445892
chin	4	0.020224	0.002367	0.015758	0.004594	-0.01833	0.000361	0.007127	0.062236	-0.00589	0.253552
chin	5	0.024223	0.000909	0.015546	0.009212	-0.01673	0.002618	0.010477	0.010661	-0.0112	0.042608
chin	6	0.027715	1.75E-05	0.016197	0.002852	-0.01829	0.000258	0.010832	0.003734	-0.00772	0.125466
chin	7	0.021963	0.000446	0.013392	0.010519	-0.01804	0.000191	0.010792	0.00273	-0.01137	0.019306
chin	8	0.022747	0.000224	0.014104	0.006513	-0.01674	0.000462	0.009774	0.006132	-0.00692	0.150245
auricle	1	0.032685	6.48E-06	0.010881	0.072564	-0.00541	0.333453	0.012838	0.002084	-0.0008	0.886582
auricle	2	0.019704	0.00475	0.001925	0.739707	-0.01686	0.001698	0.009916	0.012883	-0.0112	0.037305
auricle	3	0.015735	0.019738	0.002634	0.639698	-0.00938	0.071533	0.006192	0.1096	-0.0163	0.001813
auricle	4	0.013551	0.022693	0.000971	0.851141	-0.0133	0.004699	0.007225	0.042811	-0.01479	0.002196
auricle	5	0.008515	0.164343	0.000116	0.98232	-0.01004	0.035928	0.007105	0.048021	-0.0181	0.000196
auricle	6	0.015561	0.008342	0.00189	0.713533	-0.01376	0.003258	0.004322	0.223628	-0.01445	0.002667
auricle	7	0.03148	5.75E-06	0.009785	0.087031	-0.01044	0.049214	0.006717	0.087523	-0.00677	0.201071
crown	1	0.021711	0.000255	0.012478	0.012018	-0.01842	5.99E-05	0.010299	0.002587	-0.00325	0.480694
crown	2	0.022208	2.14E-05	0.009762	0.030246	-0.01794	1.30E-05	0.00794	0.010539	-0.00506	0.227793
crown	3	0.020721	0.000108	0.013655	0.002808	-0.01959	2.98E-06	0.007034	0.025335	-0.00643	0.130081
crown	4	0.025781	7.37E-08	0.012605	0.002368	-0.01716	5.60E-06	0.007599	0.007829	-0.00362	0.348635
crown	5	0.026143	1.01E-06	0.011385	0.012289	-0.01424	0.000645	0.01011	0.001245	-0.00729	0.084555
crown	6	0.023518	1.19E-05	0.011489	0.011784	-0.01503	0.000333	0.00907	0.003877	-0.00726	0.08716
crown	7	0.02685	2.93E-08	0.013435	0.001097	-0.01315	0.000496	0.009011	0.001469	-0.00224	0.557677
crown	8	0.023733	5.48E-07	0.011154	0.006391	-0.01031	0.005671	0.005847	0.037953	-0.00251	0.510649
eyestripe	1	0.011435	0.060338	0.008003	0.122566	-0.00019	0.968614	0.007618	0.032788	0.000988	0.837657
eyestripe	2	0.015158	0.008482	0.008952	0.076964	-0.00733	0.109333	0.007709	0.027299	-0.00147	0.756096
eyestripe	3	0.024249	2.99E-06	0.00501	0.270215	-0.01415	0.000593	0.001609	0.607758	-0.0038	0.370823
eyestripe	4	0.019225	0.000164	0.002277	0.603143	-0.01409	0.000439	0.004246	0.159419	-0.01127	0.005777
eye	1	-0.00388	0.492278	0.000736	0.878903	0.009426	0.033222	0.007401	0.026175	0.004024	0.370893
eye	2	0.009674	0.004501	0.008987	0.001504	-0.00101	0.700923	0.005405	0.005497	-0.00055	0.834962
eye	3	0.001123	0.748845	0.006244	0.034446	0.000874	0.747868	0.006613	0.001139	-0.00424	0.12171
eye	4	-0.00056	0.908312	0.008734	0.034066	0.000342	0.927879	0.00617	0.029637	0.002094	0.584782
nape	1	0.022419	2.59E-06	0.00805	0.045043	-0.01013	0.006152	0.00586	0.03399	-0.00418	0.263083
nape	2	0.024802	6.98E-07	0.007359	0.079473	-0.01254	0.001193	0.004726	0.10154	-0.00513	0.187811
nape	3	0.02548	1.13E-06	0.006748	0.131665	-0.01122	0.006176	0.004492	0.144981	-0.00863	0.038443
nape	4	0.026328	3.46E-06	0.005708	0.248107	-0.00914	0.041657	0.007753	0.022945	-0.01158	0.012084
nape	5	0.032073	9.20E-09	0.004659	0.329475	-0.01139	0.009153	0.008394	0.010778	-0.0096	0.031035
nape	6	0.032059	2.51E-07	0.00755	0.14704	-0.01553	0.001231	0.011938	0.000869	-0.00746	0.123003

Table 3 Effect sizes and p-values for the correlations between body regions and different life-history traits from the multiple predictor model for each triangle in males.

patch	patch_tri_	m.size.eff	m.size.P	m.life.eff	m.life.P	m.sex.eff	m.sex.P	m.coop.ef	m.coop.P	m.mig.eff	m.mig.P
upperBill	1	0.018332	0.000423	0.006178	0.127667	-0.00176	0.646541	-0.0049	0.078496	-0.00561	0.133345
upperBill	2	0.020898	1.13E-05	0.004361	0.254887	0.00123	0.731414	-0.00091	0.729072	-0.00141	0.689957
upperBill	3	0.023177	2.30E-08	0.010215	0.003531	-0.00177	0.58213	-0.00163	0.497409	0.001174	0.718062
upperBill	4	0.017515	7.99E-05	0.003774	0.290562	-0.00388	0.245122	0.001701	0.488229	-0.00094	0.776377
lowerBill	1	0.011203	0.023472	0.00854	0.029533	-0.0035	0.342182	-0.00019	0.944659	0.000214	0.952822
lowerBill	2	0.010346	0.025617	0.008499	0.026683	0.000994	0.779764	-6.08E-05	0.981584	0.000814	0.818712
lowerBill	3	0.014374	0.005888	0.005596	0.187915	-4.40E-06	0.999115	0.00102	0.726819	-0.00026	0.948244
lowerBill	4	0.013009	0.009355	0.006969	0.091053	0.003953	0.301961	0.001172	0.679104	0.005991	0.116874
lowerBill	5	0.013767	0.004048	0.009097	0.025705	0.004899	0.19017	0.002879	0.304937	0.003713	0.327587
lore	1	0.00209	0.739193	0.018965	0.000423	0.00778	0.113742	0.00207	0.575874	0.009228	0.065108
lore	2	-0.00021	0.973023	0.013759	0.007918	0.008459	0.076963	1.34E-05	0.996989	0.009845	0.040591
lore	3	0.002967	0.627014	0.001192	0.814733	0.004281	0.363121	0.002136	0.541729	0.004475	0.343299
lore	4	0.006757	0.266211	0.009217	0.076947	0.002066	0.664658	0.004191	0.242815	0.004748	0.327676
lore	5	-0.00265	0.667934	0.004957	0.338407	0.007514	0.116172	0.003698	0.299105	0.005372	0.26371
lore	6	-0.00481	0.479686	0.002207	0.705258	0.005201	0.32984	-0.00074	0.853209	-0.0049	0.367316
lore	7	-0.00207	0.737848	0.002694	0.609676	0.008123	0.092934	0.004249	0.242388	0.000642	0.896092
lore	8	0.000882	0.88065	0.005616	0.26802	0.008763	0.058174	0.001721	0.62218	0.001475	0.7548
chin	1	0.014364	0.010365	0.01242	0.00863	0.00667	0.125293	-0.00028	0.931192	0.009221	0.035826
chin	2	0.021603	0.003495	0.015243	0.010954	0.005549	0.321185	0.004733	0.250098	0.010918	0.0487
chin	3	0.020257	0.003892	0.020776	0.000485	0.008291	0.129231	0.001387	0.734801	0.007975	0.149317
chin	4	0.015376	0.031451	0.015599	0.006854	0.00783	0.146565	0.003831	0.333543	0.010084	0.058534
chin	5	0.014959	0.053268	0.016565	0.008259	0.012699	0.030148	0.005519	0.2001	0.008576	0.138971
chin	6	0.024866	0.000335	0.018089	0.001529	0.008886	0.093507	0.008747	0.025721	0.004124	0.435017
chin	7	0.016584	0.011974	0.014003	0.011796	0.014183	0.005624	0.008355	0.028942	-0.00444	0.38956
chin	8	0.020167	0.002551	0.015794	0.003718	0.01198	0.018241	0.007456	0.046177	0.006559	0.192546
auricle	1	0.020764	0.004402	0.018446	0.002755	0.012895	0.022869	0.00136	0.748149	0.011812	0.039041
auricle	2	0.010175	0.146303	0.004091	0.482818	0.013131	0.01497	-0.00149	0.710176	-0.00263	0.626704
auricle	3	0.003163	0.658698	0.002369	0.686676	0.014071	0.010062	0.002021	0.616715	-0.00587	0.280766
auricle	4	0.003447	0.585381	-0.00098	0.855713	0.00964	0.051307	0.003266	0.379523	-0.00616	0.219958
auricle	5	0.001177	0.854742	-0.00096	0.858956	0.007444	0.135678	0.003915	0.293396	-0.00782	0.120192
auricle	6	0.005254	0.417378	-0.00065	0.907455	0.007639	0.133066	0.000933	0.807608	-0.00613	0.23679
auricle	7	0.018944	0.011246	0.008499	0.163892	0.008729	0.124765	0.005679	0.175948	0.002303	0.683517
crown	1	0.008784	0.176846	0.015341	0.004761	0.003852	0.442844	0.008364	0.025206	0.002514	0.61793
crown	2	0.013346	0.023686	0.009661	0.05572	-3.55E-05	0.993876	0.003183	0.359781	0.003097	0.50983
crown	3	0.011091	0.063207	0.01395	0.005387	0.000409	0.929431	0.00554	0.107948	0.003394	0.465597
crown	4	0.018727	0.000674	0.012562	0.007762	-3.45E-06	0.999363	0.005445	0.093683	0.00426	0.331925
crown	5	0.018499	0.001163	0.013567	0.004612	0.006423	0.145411	0.007926	0.01612	0.001076	0.808682
crown	6	0.016219	0.005762	0.011293	0.023378	0.003311	0.469147	0.008033	0.019131	-0.00074	0.872622
crown	7	0.018062	0.001075	0.010334	0.030214	0.003586	0.409322	0.008946	0.00648	0.003428	0.440319
crown	8	0.011314	0.039298	0.008761	0.061223	0.007129	0.096598	0.006528	0.042786	0.00369	0.396693
eyestripe	1	0.007768	0.195631	0.009106	0.079575	0.011482	0.0153	0.008243	0.021307	0.003837	0.428
eyestripe	2	0.008267	0.16087	0.013378	0.009153	0.009314	0.045852	0.007051	0.046232	0.000556	0.907438
eyestripe	3	0.007601	0.168139	0.013154	0.00706	0.010549	0.016646	0.003379	0.315744	0.002207	0.628675
eyestripe	4	0.008825	0.112354	0.00483	0.314206	0.010445	0.017062	0.002414	0.465309	-0.00135	0.763177
eye	1	-0.00449	0.4159	0.006821	0.144511	0.005702	0.184347	0.000118	0.970772	0.004078	0.347984
eye	2	0.004483	0.201893	0.005718	0.048014	0.001516	0.572378	0.002659	0.180897	-0.0018	0.50248
eye	3	-0.00061	0.869671	0.006922	0.024931	0.004264	0.13599	0.005409	0.01083	0.000901	0.752771
eye	4	-0.00545	0.24526	0.006376	0.110367	0.006106	0.095595	0.001005	0.714683	0.003311	0.372721
nape	1	0.013857	0.009695	0.01167	0.009354	0.010192	0.01392	0.006689	0.030313	0.007234	0.082616
nape	2	0.018649	0.001004	0.008929	0.057167	0.010095	0.020361	0.006011	0.062588	0.002001	0.645662
nape	3	0.01498	0.012761	0.00735	0.146809	0.013088	0.005058	0.004287	0.218682	-0.00055	0.906269
nape	4	0.014565	0.020735	0.007236	0.177938	0.013731	0.005309	0.00509	0.168703	-0.00222	0.656614
nape	5	0.020825	0.000791	0.002151	0.678626	0.01671	0.000496	0.005315	0.136687	-0.00845	0.079689
nape	6	0.023549	0.000608	0.007261	0.194377	0.009606	0.065538	0.007291	0.057981	-0.00457	0.377512