ROLE OF NATURAL AND SEXUAL SELECTION IN THE EVOLUTION OF BUTTERFLY WING COLOURS

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DECLARATION

I hereby declare that this thesis is my original work (except for the behavioural experiment in Chapter 5 which was done by Dr. Krushnamegh Kunte of the National Centre for Biological Sciences, Bangalore, India) and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Su Shiyu
5 August 2015

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SUMMARY

Butterfly wing colours have various adaptive functions such as camouflage, aposematism, mimicry, species recognition, and mate choice. Due to their multifunctional nature, butterfly wing colours are likely to experience two or more selection pressures simultaneously. Thus, the primary aim of this thesis is to study how natural and sexual selection have shaped the evolution of butterfly wing colours. Natural selection has led to the evolution of mimicry in several butterfly species. However, assessments of mimicry in the past were largely based on human vision, which differs considerably from that of birds, which are presumably the intended receivers and selective agents of butterfly mimicry. Thus, I analyzed mimetic resemblance of butterfly mimicry rings found in the Western Ghats of India from an avian visual perspective. Females of sexually monomorphic mimetic species were better Batesian mimics than males, suggesting stronger natural and sexual selection on females and males, respectively. Female-limited and sexually monomorphic mimics showed equally good mimetic resemblance. Also, ventral wing surfaces exhibited greater mimetic resemblance than dorsal wing surfaces, implying stronger natural selection on ventral colouration and/or antagonistic sexual selection on dorsal colours. Therefore, natural and sexual selection pressures influenced butterfly mimetic resemblance in a sex- and wing surface-specific manner. Sexual dimorphism is often treated as an indicator of sexual selection. However, I found that sexual dichromatism in a group of swallowtail butterflies was closely associated with female-limited mimicry, suggesting that natural selection was the main driver of sexual dimorphism in these butterflies. Furthermore, modeling of avian colour vision revealed that mimetic butterflies displayed adaptive wing colour changes that were not apparent to human observers. Also, butterflies with sexually monomorphic wing patterns were sexually dimorphic in brightness to conspecifics and predators. These findings indicated that it is important to analyze animal colours using the perception of the relevant receivers. Previous studies of butterfly mate choice have often used surrogate measures of mate preference. By conducting mating assays, I showed that female *Papilio polytes* butterflies did not prefer to mate with males having brighter dorsal white patches, suggesting that sexual dimorphism in brightness was not due to directional selection by females. Males preferred to mate with mimetic females than with non-mimetic ones, implying that the mimetic female form was favoured by both natural and sexual selection. Sexual selection has been implicated in the evolution of female-limited mimetic polymorphism in butterflies. Behavioural experiments showed that female *Papilio alphenor* butterflies did not discriminate between males with the natural phenotype and those with altered phenotype, suggesting that male wing colouration was not under stabilizing sexual selection. Males mated preferentially with mimetic females, indicating that persistence of the non-mimetic female form was not due to male mate choice. Hence, female-limited mimetic polymorphism probably resulted from stronger natural selection on females and the negative frequency-dependent advantage of Batesian mimicry. Overall, my findings demonstrated that butterfly wing colours have evolved under both natural and sexual selection pressures that show intra- and intersexual differences, and also highlighted the importance of considering receiver vision in studies of signal evolution.

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CHAPTER 1

GENERAL INTRODUCTION

Animal colours have fascinated and intrigued human observers for centuries. The study of animal colours has contributed tremendously to our understanding of the production, perception, and evolution of visual signals. The adaptive significance of colouration has been studied extensively in animals such as birds (Hill & McGraw, 2006a, 2006b). Among invertebrates, butterflies have attracted the most attention and scientific interest due to their large, conspicuous, and often colourful wings. Butterflies (Papilionoidea) are charismatic insects that belong to the order Lepidoptera, which also includes moths. Although the family-level phylogenetic relationships are not fully resolved (Heikkilä *et al.*, 2012; Regier *et al.*, 2013; Kawahara & Breinholt, 2014; Kim *et al.*, 2014), at least four butterfly families are commonly recognized, including Papilionidae, Pieridae, Nymphalidae, and Lycaenidae.

Butterflies are excellent subjects for the study of the adaptive significance of colouration for various reasons. First, they show a high diversity of wing colour patterns that rank among the brightest and most chromatic found in animals. Second, many species are easily reared in captivity, with relatively short generation times (i.e., a few weeks) which are conducive for genetic inquiries of wing colour development (Kemp & Rutowski, 2007; Talloen *et al.*, 2009). Third, their holometabolous life cycle allows the investigation of how and when resources are acquired and allocated to wing colouration (Burghardt *et al.*, 2001; Talloen *et al.*, 2004; Kemp *et al.*, 2006; Kemp, 2008a; Pegram *et al.*, 2013). Lastly, butterflies are behaviourally suitable for experimental and observational studies in the field as well as the laboratory, and their wing colour traits can be precisely manipulated using simple techniques (e.g., Robertson & Monteiro 2005).

Mechanisms of Colour Production

The colours on butterfly wings reside in the rows of chitinous scales that cover the wing membrane like tiles on a roof. There are normally two types of scales: the basal scales, which lie directly above the wing membrane, and the cover scales, which overlay the basal scales. Both types of scales are approximately 100 μm long, and cover scales are generally more specialized in shape than basal scales, which are usually rectangular (Ingram & Parker, 2008). The structure of a typical wing scale is shown in Figure 1.1. Each scale is basically a dorsoventrally flattened sac with an upper and lower surface (known as lamina) connected by pillar-like trabeculae (Ingram & Parker, 2008). The lower lamina is generally smooth and flat, while the upper lamina has a more elaborate structure which typically consists of rows of longitudinal ridges (Ingram & Parker, 2008; Ghiradella, 2010). These ridges are composed of slanted and overlapping lamellae, with microribs running between and perpendicular to the lamellae (Ghiradella, 1991, 2010). The parallel ridges are joined at intervals by cross-ribs, and together they frame a series of windows that open into the scale interior or lumen (Ghiradella, 1991, 2010).

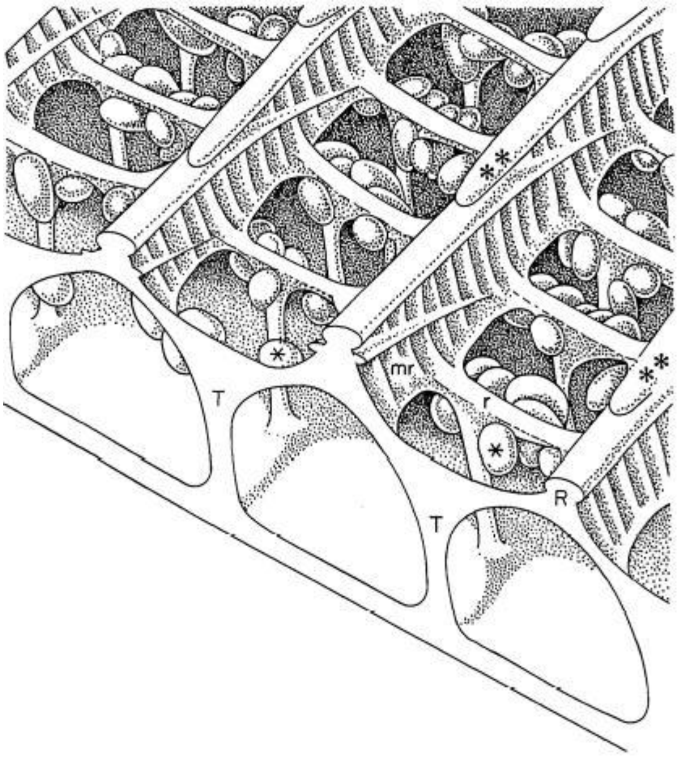

Figure 1.1. Diagram of a butterfly wing scale showing the lower lamina, trabeculae (T), ridges (R), lamellae (**), microribs (mr), and cross-ribs (r). Pigment granules (*) are present only in the scales of pierid butterflies. Taken from (Ghiradella, 1991).

Butterfly wing colours are traditionally categorized into two types: pigmentary and structural. Pigmentary colours result from the deposition of chemical pigments in scales that selectively absorb specific wavelengths of light. On the other hand, structural colours are produced when physical interaction between light and scale nanostructures (e.g., ridge lamellae or microribs) leads to the reflection of specific wavelengths of light.

Pigmentary colours

Several classes of chemical pigments are known to contribute to butterfly wing colouration. All black pigments and the vast majority of brown pigments in butterfly wings are melanins, which are complex polymers synthesized from the amino acid tyrosine (Koch & Kaufmann, 1995; Ferguson *et al.*, 2011). Other than melanins, the various butterfly families also express characteristically different pigment classes. For instance, in butterflies from the family Papilionidae, various types of papiliochromes, which are formed from the precursor kynurenine, yield colours ranging from pale yellow to red (Umebachi, 1977, 1985; Koch *et al.*, 2000a; Stavenga *et al.*, 2014a). Kynurenine is also used by nymphalid butterflies to create another class of pigments known as ommochromes, which are yellow, red, or brown pigments (Nijhout, 1997; Reed & Nagy, 2005; Stavenga *et al.*, 2014b). On the other hand, white, yellow, and orange colours in pierid butterflies are produced by pterins (such as leucopterin, xanthopterin, and erythropterin) which are derived from guanosine triphosphate (Morehouse *et al.*, 2007; Wijnen *et al.*, 2007). Pterin pigments are deposited in granular beads attached to the cross-ribs in the wing scales of pierid butterflies (Giraldo & Stavenga, 2007; Morehouse *et al.*, 2007), whereas melanins, papiliochromes, and ommochromes are distributed throughout the scale components. Flavonoids, which are plant pigments, are generally uncommon but widespread, occurring in some species in different butterfly families (Wilson, 1985, 1986, 1987; Burghardt *et al.*, 1997; Geuder *et al.*, 1997). Bile pigments and carotenoids have also been found in the wings of some butterfly species (Barbier, 1981; Rothschild & Mummery, 1985).

Structural colours

When the scale structures (e.g., ridge lamellae or microribs) are irregularly organized, incident light is scattered randomly and the scales are white in the absence of pigment. However, when the scale components have a regular, periodic arrangement, structural colours ranging from ultraviolet to blue and green are produced. Periodic nanostructures of chitin and air in the scales enhance the reflection of certain wavelengths by constructive interference and suppress reflection of other wavelengths by destructive interference. In many butterfly species, structural colours are caused by multilayers composed of vertical stacks of alternating layers of chitin and air (Ingram & Parker, 2008). For instance, the famous *Morpho* butterflies and many pierid butterflies have scales whose ridges bear lamellae that function as multilayers to produce colours ranging from ultraviolet to blue (Vukusic *et al.*, 1999; Kinoshita *et al.*, 2002; Rutowski *et al.*, 2007; Wilts *et al.*, 2011). On the other hand, the blue colouration of many lycaenid butterflies is due to perforated multilayers in the scale lumen (Wilts *et al.*, 2009; Bálint *et al.*, 2012). The scale lumen of some lycaenids contain three-dimensional (3D) photonic crystals, composed of a matrix of chitin with regularly arranged spherical air spaces, which reflect blue and yellow wavelengths to result in a green colouration (Kertész *et al.*, 2006; Prum *et al.*, 2006; Michielsen & Stavenga, 2008; Michielsen *et al.*, 2010). Similar 3D structures occur in papilionid butterflies (Prum *et al.*, 2006; Poladian *et al.*, 2009; Saranathan *et al.*, 2010), which also produce structural colours using thin-film multilayers in the scale lumen or lamina (Vukusic *et al.*, 2001; Kolle *et al.*, 2010; Wilts *et al.*, 2012a).

Combination of pigmentary and structural colours

Pigmentary and structural colouration mechanisms often simultaneously contribute to the wing colouration. For instance, in the pierid butterfly *Colias eurytheme*, the presence of pterin pigments, which strongly absorb short wavelengths, amplifies the iridescent properties of the male’s UV reflectance, which is structural in origin and functions as a sexual signal (Rutowski *et al.*, 2005). The wings of butterflies of the *Papilio nireus* group have conspicuous blue-green bands surrounded by black margins. Papiliochrome pigments in the upper lamina function as a violet-absorbing long-pass optical filter, thus shifting the peak reflectance of multilayers in the lower lamina to blue-green wavelengths, and also reduce the iridescence of the reflectance to produce an angle-independent blue-green colour (Wilts *et al.*, 2012a). Similar spectral tuning occurs in *Parides sesostris*, whereby the filtering effect of papiliochrome pigments in the upper lamina shapes the reflectance of the underlying photonic crystals (Wilts *et al.*, 2012b). The brilliant structural blue colouration of *Morpho* wings is achieved by the presence of melanin pigments in the scales, which prevent backscatter by absorbing most of the transmitted light, hence enhancing the spectral purity of the reflectance (Kinoshita *et al.*, 2008). Removal of melanin results in a whitish wing colour, which demonstrates that the blue colouration of *Morpho* butterflies is created by a combination of pigmentary and structural mechanisms (Yoshioka & Kinoshita, 2006).

Functions of Butterfly Wing Colours

Butterfly wing colours have three adaptive purposes: thermoregulation, predator protection, and intraspecific communication.

Thermoregulation

Butterflies require elevated thoracic temperatures for flight (Watt, 1968; Rawlins, 1980; Kingsolver, 1983), and being ectothermic, they achieve these temperatures by basking in the sun. Butterfly wing colours, particularly the colouration of the basal wing area, influence the efficiency of solar energy absorption (Wasserthal, 1975). As dark colours absorb heat more efficiently than light colours, the degree of wing melanization affects thermoregulation and is expected to be adapted to local environmental conditions. For instance, butterflies found in colder environments, such as higher latitudes or altitudes, typically show greater wing melanization compared to conspecifics living in warmer habitats (Watt, 1968; Kingsolver, 1983; Guppy, 1986; Ellers & Boggs, 2002; Biró *et al.*, 2003; Karl *et al.*, 2009; Tuomaala *et al.*, 2012). The adaptive significance of variation in wing melanization along an elevational gradient was demonstrated using transplant experiments and experimental manipulation of wing colour in *Colias* butterflies (Ellers & Boggs, 2004). Similarly, seasonal polyphenism of wing melanization in many pierid butterflies represents a form of adaptive phenotypic plasticity for effective thermoregulation, with butterflies flying during spring having more basal melanin than those flying in summer (Kingsolver & Wiernasz, 1991; Kingsolver, 1995a, 1995b; Stoehr & Goux, 2008). Increasing basal wing melanization experimentally reduced the survival likelihood of the butterfly *Pontia occidentalis* during hot summer conditions, presumably due to the greater risk of overheating (Kingsolver, 1996).

Anti-predation

Camouflage

In most butterflies, the ventral wings, exposed when the butterfly is at rest, have a dull inconspicuous colour (e.g., brown) which serves as a means of camouflage by background matching. For instance, the dry-season form of *Bicyclus anynana* has a brown ventral colouration which is highly cryptic against brown leaf litter, a typical background in the dry season (Lyytinen *et al.*, 2004). In many lycaenid butterflies such as *Callophrys rubi* and *Cyanophrys remus*, the green colouration of their ventral wings resembles that of plant leaves (Kertész *et al.*, 2006; Biró *et al.*, 2007; Michielsen *et al.*, 2010). Similarly, the silvery white colour of the ventral wings of *Curetis acuta* facilitates camouflage when the butterfly is resting in a shaded, foliaceous environment (Wilts *et al.*, 2013). Unlike background matching, disruptive colouration hinders prey detection or recognition by using bold contrasting markings on the prey's periphery to break up its outline (Stevens & Cuthill, 2006; Stevens & Merilaita, 2009a). Disruptive colouration has been shown to have a protective effect against avian predators (Cuthill *et al.*, 2005; Schaefer & Stobbe, 2006; Stevens *et al.*, 2006). Artificial butterflies that exhibited stripes with contrasts similar to those of the butterfly species *Limenitis camilla* survived equally well as background-matching butterflies without the stripes (Stobbe & Schaefer, 2008), suggesting that disruptive colouration and background matching could provide comparable protection against predation. Another form of camouflage is masquerade, where prey recognition is prevented by resemblance to an inedible object (Stevens & Merilaita, 2009b). For example, the ventral wing surfaces of *Kallima* butterflies mimic dead leaves and have colours that are indistinguishable from those of actual dead leaves to birds (Stoddard, 2012).

Eyespots

Comprising of concentric rings of contrasting colours, eyespots are common markings found on the wings of many butterfly species, especially nymphalids. They are thought to be anti-predatory through two main mechanisms, depending on their size and position on the wings (reviewed by Stevens 2005; Kodandaramaiah 2011). Large and conspicuous eyespots on dorsal wing surfaces are believed to intimidate predators, and have been shown to increase prey survival by reducing the probability of attack on prey possessing such eyespots (Vallin et al. 2005; Stevens et al. 2007, 2008; Kodandaramaiah et al. 2009; Merilaita et al. 2011; but see Pinheiro et al. 2014). On the other hand, smaller eyespots found on ventral wing margins are suggested to divert the attacks of predators toward themselves, allowing the butterfly to escape with a torn wing. Although earlier studies did not find support for this hypothesis (Lyytinen *et al.*, 2003, 2004; Vlieger & Brakefield, 2007), more recent research found that ventral eyespots are able to misdirect avian predators under certain environmental conditions (Olofsson *et al.*, 2010, 2013; Vallin *et al.*, 2011). Recent work also suggest that the protective function of ventral eyespots may be more effective against invertebrate predators (Prudic *et al.*, 2015). In addition, many lycaenid butterflies possess an eyespot at the anal angle of the ventral hindwing; such eyespots are known as “false heads” (Robbins, 1980). There is evidence that “false heads” deflect predatory attacks away from the true head, thereby increasing the probability of escape and survival of the butterfly (Robbins, 1981; Wourms & Wasserman, 1985; Tonner *et al.*, 1993; Sourakov, 2013).

Aposematism and mimicry

Some butterfly species sequester toxic secondary compounds from their larval host plants as a form of chemical defense against predation (reviewed by Nishida 2002). Wing colours of these unpalatable butterflies could then function as aposematic signals that encourage predators to learn to avoid attacking them (Ruxton *et al.*, 2004a). Behavioural studies suggest that avian predators are able to recognize unpalatable butterflies by their wing colouration and consequently avoid them (Chai, 1986, 1988; Mallet & Barton, 1989; Codella & Lederhouse, 1990; Langham, 2004, 2006; Merrill *et al.*, 2012). Furthermore, many unpalatable species may converge on the same colour pattern to share the mortality costs of educating predators, a phenomenon known as Müllerian mimicry (Müller, 1879). This form of visual mimicry is exemplified by neotropical *Heliconius* butterflies and their co-mimics (Brown & Benson, 1974; Mallet & Gilbert, 1995; Joron *et al.*, 1999). The adaptive significance of Müllerian mimicry has been proven experimentally in the field (Benson, 1972; Kapan, 2001; Pinheiro, 2003). In addition, some palatable butterfly species have evolved wing colour patterns that are highly similar to those of sympatric unpalatable species; this is known as Batesian mimicry (Bates, 1862). Due to their resemblance to toxic species, palatable Batesian mimics gain some degree of protection from predators, as demonstrated by some studies (Brower, 1958; Platt *et al.*, 1971; Codella & Lederhouse, 1989; Uésugi, 1996; Prudic *et al.*, 2002).

Intraspecific communication

Butterfly wing colours play an important role in intraspecific communication (Kemp & Rutowski, 2011). First, wing colouration may act as visual signals for mate location and identification. For instance, several pierid butterfly species utilize sexual dimorphism in UV reflectance (present in males and absent in females) for sexual discrimination (Rutowski, 1977, 1981; Silberglied & Taylor, 1978). Wing colour patterns have also been shown to be used for conspecific mate recognition between closely related butterfly species (Silberglied & Taylor, 1978; Wiernasz & Kingsolver, 1992; Jiggins *et al.*, 2001; Fordyce *et al.*, 2002; Sweeney *et al.*, 2003; Kronforst *et al.*, 2006). Second, males and females may discriminate among potential mates based on intrasexual variation in wing colouration. Female mate choice of several butterfly species is influenced by male wing colour, with females preferring to mate with males having brighter and/or more chromatic colouration (Robertson & Monteiro, 2005; Davis *et al.*, 2007; Kemp, 2007, 2008b; Papke *et al.*, 2007; Morehouse & Rutowski, 2010; Rutowski & Rajyaguru, 2012). Similarly, female wing colour patterns may affect the mating preferences of male butterflies (Emmel, 1972; Wiernasz, 1995; Burghardt *et al.*, 2000; Knüttel & Fiedler, 2001; Ellers & Boggs, 2003; Kemp & Macedonia, 2007; Chamberlain *et al.*, 2009; Prudic *et al.*, 2011). Moreover, male butterflies of many species exhibit territorial behaviour and hence wing colour patterns may also be involved in male-male interactions (Silberglied, 1984). However, this hypothesis has rarely been tested and experimental evidence is lacking (but see Lederhouse & Scriber 1996).

Interactions among functions

As butterfly wing colours have multiple adaptive functions, they may be subject to two or more selective pressures simultaneously. In *Heliconius* butterflies, mimetic wing patterns that serve as aposematic signals are also involved in mate choice, leading to assortative mating between closely related species (Jiggins *et al.*, 2001, 2004; Naisbit *et al.*, 2001; Kronforst *et al.*, 2006, 2007; Mavárez *et al.*, 2006; Munoz *et al.*, 2010; Merrill *et al.*, 2011). Furthermore, hybrids produced from interspecific mating experience greater predation risk (Merrill *et al.*, 2012) and lower mating success (Naisbit *et al.*, 2001; Munoz *et al.*, 2010) as compared to the parental species. Therefore, natural and sexual selective pressures acting on *Heliconius* wing colouration appear to be working in the same direction. However, convergence in wing patterns between co-mimetic *Heliconius* species may interfere with mate recognition and lead to interspecific attraction (Estrada & Jiggins, 2008). In non-mimetic butterflies, wing colour traits that make males more attractive to females might also increase their predation risk. For instance, female *Pieris rapae* butterflies prefer to mate with more chromatic males, which are more conspicuous to avian predators (Morehouse & Rutowski, 2010). In addition, females of several butterfly species show mating preferences for males with brighter UV reflectance (Robertson & Monteiro, 2005; Kemp, 2007, 2008b; Papke *et al.*, 2007), but these preferred males could be more easily detected by visually oriented diurnal predators (Lyytinen, 2004). Similarly, male mate choice may act against adaptive wing colour changes in female butterflies. For example, female *Colias philodice* butterflies show increased wing melanization with increasing elevation as a form of thermoregulatory adaptation (Ellers & Boggs, 2004). However, males at all elevations prefer females with less melanization than heavily-melanized females, indicating a conflict between natural and sexual selection on female wing colouration at higher elevations (Ellers & Boggs, 2003).

Study Objectives

As a result of their multifunctional nature, butterfly wing colours are the evolutionary outcome of various selective pressures acting simultaneously on them. Therefore, the primary aim of this study is to gain a better understanding of how natural and sexual selection pressures have shaped the evolution of butterfly wing colours. Four main objectives are highlighted in the following chapters. First, due to differences in behaviour, morphology, and life history, selective pressures may act differentially on males and females, as well as on the dorsal and ventral wing surfaces. Thus, I investigated how wing colouration in a diverse community of mimetic butterflies has evolved to affect mimetic resemblance in a sex- and wing surface-specific manner (Chapter 2). Next, I examined whether natural selection for Batesian mimicry has resulted in adaptive wing colour changes in a group of swallowtail butterflies, and also whether butterflies with sexually monomorphic wing patterns show sexual dichromatism (Chapter 3). In both chapters, wing colouration of butterflies were quantified objectively to avoid the biases of human vision, and subsequently analysed from the visual perspective of the intended receivers. Then, I studied whether sexual dichromatism in a *Papilio* butterfly species was driven by sexual selection in the form of female mate choice, and also whether there is a conflict between natural and sexual selection acting on female wing colouration (Chapter 4). In Chapter 5, I determined the role of sexual selection in the evolution of female-limited mimetic polymorphism using another species of *Papilio* butterfly. Finally, I summarized the main findings of this study and proposed promising areas for future research (Chapter 6).

CHAPTER 2

BUTTERFLY MIMETIC RESEMBLANCE FROM AN AVIAN VISUAL PERSPECTIVE

ABSTRACT

Mimicry imposes strong selection pressure on butterfly wing colour patterns, which also serve other functions such as sexual signalling. Since insectivorous birds exert arguably the strongest selection on butterfly mimicry, mimetic resemblance needs to be assessed through avian visual perception, which differs considerably from that of humans. Here I investigated and quantified butterfly mimetic resemblance from an avian visual perspective using Indian butterfly mimicry rings. Reflectance spectrometry provided an objective measurement of the wing colouration of mimetic butterflies and their models, and a physiological model of avian colour vision was then applied to analyse whether the wing colours of models and mimics were discriminable from a predator's perspective. Intra- and interspecific comparisons of mimetic resemblance were subsequently made to test specific predictions. As predicted, I found that female butterflies were better Batesian mimics than males, indicating stronger natural selection on females and/or antagonistic sexual selection on males. Contrary to expectation, female-limited mimics and sexually monomorphic mimics were equally good Batesian mimics, implying that in female-limited mimetic species, intersexual genetic correlations between mimetic and non-mimetic wing patterns did not hamper sex-specific selection for mimicry. Also, mimetic resemblance was greater on the ventral wing surface than on the dorsal surface, suggesting stronger natural selection on ventral colouration and/or antagonistic selection pressure on dorsal colours. These results show that mimetic resemblance in butterfly mimicry rings has evolved under various selective forces acting in a sex- and wing surface-specific manner. I discussed the evolutionary significance of my findings and proposed potential areas for future research.

INTRODUCTION

Mimicry, which involves the adaptive resemblance in signal between two or more species, provides some of the most compelling examples of natural selection (Ruxton *et al.*, 2004a). Two classical forms of mimicry, Batesian and Müllerian, have been recognized traditionally (but see Ruxton *et al.*, 2004b; Balogh *et al.*, 2008). Batesian mimicry occurs when a palatable species (the mimic) gains a degree of protection from predators by resembling an unpalatable species (the model) (Bates, 1862). Due to the parasitic relationship between mimics and models, Batesian mimics typically benefit most when they are rare relative to their models (Brower, 1960; Huheey, 1980; Lindström *et al.*, 1997), and hence experience negative frequency-dependent selection, which can lead to mimetic polymorphism (Clarke & Sheppard, 1960, 1972; Brower & Brower, 1962; West, 1994). Müllerian mimicry, on the other hand, occurs when two unpalatable species (known as co-mimics) benefit from mutual resemblance by sharing the mortality costs of educating predators (Müller, 1879). In contrast to Batesian mimicry, the mutualistic relationship among Müllerian co-mimics predicts positive frequency-dependent selection and mimetic monomorphism (Müller, 1879; Turner, 1987). As a result of both processes, several sympatric prey species may converge on the same mimetic signal, forming a mimicry ring which consists of one or more unpalatable species acting as models for palatable Batesian mimics (Owen, 1974; Papageorgis, 1975; Mallet & Gilbert, 1995; Beccaloni, 1997a).

Although the evolution of mimicry is driven by natural selection, animal visual signals generally reflect an interaction between natural and sexual selection pressures which act simultaneously on organisms (Ellers & Boggs, 2003; Godin & McDonough, 2003; Stuart-Fox *et al.*, 2004; Crothers *et al.*, 2011; Nokelainen *et al.*, 2012; Lindholm *et al.*, 2014; Marshall & Stevens, 2014). Mimicry has been described and studied extensively in Neotropical butterfly species (Papageorgis, 1975; Chai, 1986; Mallet & Gilbert, 1995; Beccaloni, 1997a, 1997b; Pinheiro, 2003). In mimetic butterflies, wing colour patterns which are involved in mimicry may also be used for other functions such as intraspecific communication. For instance, many closely related species of *Heliconius* butterflies, which are distasteful and exhibit Müllerian mimicry, differ in mimetic patterns and use their mimetic wing colouration in mate recognition, leading to assortative mating (Jiggins *et al.*, 2001, 2004). Conversely, interspecific courtship interference between co-mimetic *Heliconius* butterfly species has been observed, suggesting that mimetic convergence may lead to reduced efficiency in mate recognition (Estrada & Jiggins, 2008). In addition, strong sexual selection against male phenotypic changes has been implicated in the evolution of female-limited Batesian mimicry in butterflies (Turner, 1978; Krebs & West, 1988; Lederhouse & Scriber, 1996). Since butterfly wing colours serve several functions, the degree of mimetic resemblance shown by mimics would thus be determined by the interaction between multiple evolutionary pressures acting on wing colouration, especially if natural and sexual selection favour different phenotypes.

Being the primary predators of butterflies, insectivorous birds exert arguably the strongest selection on the evolution of butterfly mimetic resemblance (Chai, 1996; Pinheiro, 2003, 2011; Langham, 2004, 2006). However, assessments of butterfly mimicry are frequently based on human perception, which could be incorrect or misleading due to differences in visual capabilities between humans and birds (Bennett *et al.*, 1994). Unlike humans, birds are sensitive to ultraviolet wavelengths (300 to 400 nm; Bennett & Cuthill, 1994; Cuthill *et al.*, 2000) and most species possess tetrachromatic colour vision (compared to trichromacy in humans; Maier & Bowmaker, 1993; Osorio *et al.*, 1999). Furthermore, avian cone photoreceptors contain pigmented oil droplets which act as long-pass cut-off filters (Bowmaker *et al.*, 1997; Hart *et al.*, 1998, 2000a, 2000b). By absorbing short wavelengths below a critical value, oil droplets narrow the spectral sensitivity functions of cones, leading to a reduction in spectral overlap which improves colour discriminability and enhances colour constancy (Vorobyev *et al.*, 1998; Vorobyev, 2003; Stavenga & Wilts, 2014). As a result of these differences, birds are able to perceive a greater range and diversity of colours than do humans, making human assessment an inaccurate representation of avian perception (Cherry & Bennett, 2001; Eaton, 2005; Hastad & Odeen, 2008; but see Seddon *et al.*, 2010). To avoid the biases of human vision, recent studies involving mimicry (e.g., Avilés, 2008; Cheney & Marshall, 2009; Spottiswoode & Stevens, 2010; Langmore *et al.*, 2011; Antonov *et al.*, 2012; De Mársico *et al.*, 2012; Honza *et al.*, 2014) have employed the use of reflectance spectrometry to quantify animal colouration objectively (Endler, 1990), followed by visual modeling techniques that incorporate the photoreceptor spectral sensitivities of the intended receivers (Vorobyev & Osorio, 1998; Endler & Mielke, 2005).

In this study, I assessed mimetic resemblance shown by a community of Indian butterflies from an avian visual perspective. Wing colours of mimics and models were measured objectively using reflectance spectrometry over the range of bird-sensitive wavelengths (i.e., 300 to 700 nm), and mimetic resemblance was subsequently quantified using a physiological model of avian colour vision. Intra- and inter-specific comparisons of mimetic resemblance were then made to test the following predictions. Firstly, butterfly species with sexually monomorphic Batesian mimicry may possess sexual colour differences which are not apparent to the human eye. As there is evidence to suggest that female butterflies experience greater predation risk than male butterflies (Ohsaki, 1995; Ide, 2006), I predicted that females of sexually monomorphic mimetic species would be better Batesian mimics than their male counterparts. Secondly, in female-limited mimetic species, only the female is a Batesian mimic while the male shows non-mimetic ancestral colouration (Kunte, 2008). As sex-specific selection on wing colouration may be hampered by intersexual genetic correlations which prevent the independent evolution of mimetic and non-mimetic sexes (Lande, 1987), female-limited mimics were predicted to show lower mimetic resemblance than sexually monomorphic mimics. Thirdly, aerially-hunting insectivorous birds are considered to be the primary selective agents of butterfly mimicry (Chai, 1996; Pinheiro, 2003, 2011; Langham, 2004, 2006). Since dorsal wing surfaces of butterflies are exposed and visible to avian predators during flight, I predicted that dorsal wing surfaces should show better mimetic resemblance than ventral wing surfaces. The evolutionary significance of these findings was then discussed.

METHODS

Study System

The Western Ghats is a 1600-km-long mountain chain running parallel to the west coast of peninsular India, covering a geographical area of ~16 million ha and ranging in elevation from sea level to ~2700 m. Together with Sri Lanka, it forms one of the global biodiversity hotspots (Myers *et al.*, 2000; Mittermeier *et al.*, 2011). There are seven butterfly mimicry rings in the Western Ghats (Kunte 2000; Figure 2.1). These mimicry rings are named after the most common model species in each ring (e.g., [*Pachliopta*] *aristolochiae* or [*Danaus*] *genutia*), and when more than one model species is common, after the genus of the predominant models (e.g., *Tirumala*). In total, there are 14 models, all of which appear to have sexually monomorphic wing colour patterns, and 12 Batesian mimics, out of which four show sexually monomorphic mimicry while the remaining eight exhibit female-limited mimicry (i.e., females are mimetic and males are non-mimetic). Model-mimic relationships in all mimicry rings are shown in Figure 2.1 and Table 2.1. Some of the model-mimic relationships have experimental support (Smith, 1973, 1976; Uésugi, 1995, 1996; Larsen, 2006; Gordon *et al.*, 2010); the rest were established based on knowledge of the natural history and plant chemistry of larval host plants, as well as the behaviour and phenotypes of butterfly larvae and adults (Wynter-Blyth, 1957; Kunte, 2000).

Butterfly Specimens

Butterfly specimens used in reflectance spectrometry were collected from various parts of Western Ghats. The majority of these specimens were held in the Research Collections facility at the National Centre for Biological Sciences (Bangalore, India); some butterflies were also measured at the Natural History Museum (London). In all cases, I used only specimens in good condition (i.e., no extensive wing damage). Due to the large number of species involved, I measured a maximum of five specimens per sex per species; exceptions were *Pachliopta aristolochiae*, *P. hector* and *Papilio polytes romulus* (f. *stichius* and *romulus*), where 20 individuals each were measured (Table 2.1).

Table 2.1. Number of specimens measured for each butterfly species in this study

Mimicry ring	Butterfly species	Model/Mimic	No. of males	No. of females
chrysippus	*Danaus c. chrysippus*	model	5	5
	*Hypolimnas misippus**	mimic	N.A.	4
Delias	*Delias eucharis*	model	5	5
	Prioneris sita	mimic	2	1
hector	*Pachliopta hector*	model	10	10
	Papilio polytes romulus f. *romulus**	mimic	N.A.	20
aristolochiae	*Pachliopta pandiyana*	model	1	5
	Pachliopta a. aristolochiae	model	10	10
	Papilio polytes romulus f. *stichius**	mimic	N.A.	20
genutia	*Danaus g. genutia*	model	3	4
	Cethosia mahratta	model	2	3
	*Elymnias caudata**	mimic	N.A.	4
	*Argynnis hybrida**	mimic	N.A.	1
Euploea	*Euploea c. core*	model	5	5
	Euploea klugii kollari	model	1	5
	Euploea sylvester coreta	model	5	5
	*Hypolimnas bolina jacintha**	mimic	N.A.	5
	Papilio c. clytia f. *clytia*	mimic	1	4
	Papilio dravidarum	mimic	2	2
Tirumala	*Tirumala limniace exoticus*	model	5	1
	Tirumala septentrionis dravidarum	model	5	5
	Parantica a. aglea	model	3	2
	Parantica nilgiriensis	model	5	1
	Papilio c. clytia f. *dissimilis*	mimic	2	4
	*Pareronia c. ceylanica**	mimic	N.A.	1
	*Pareronia hippia**	mimic	N.A.	5

N.A., not available because males are non-mimetic in species showing female-limited mimicry (marked with *)

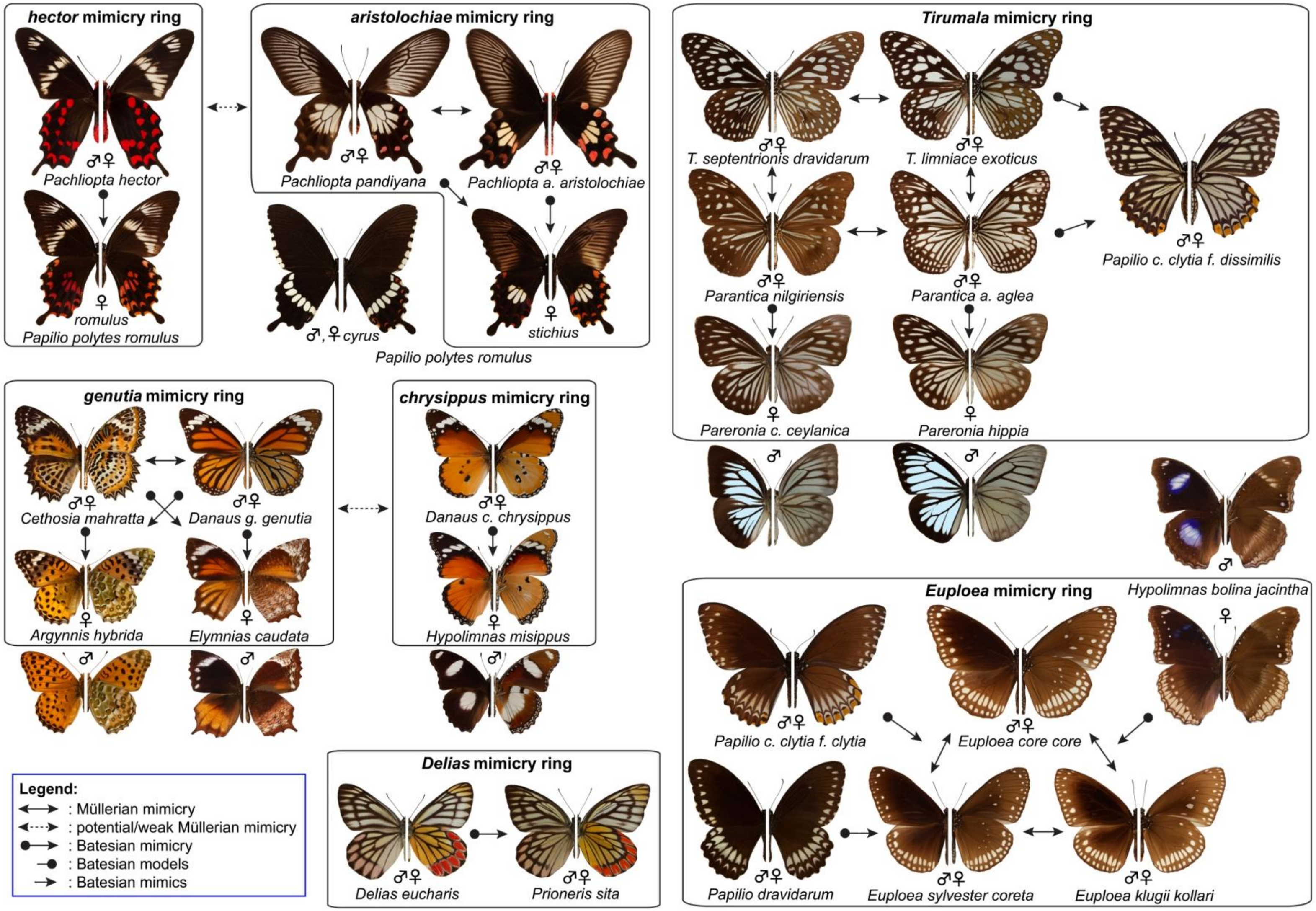

Figure 2.1. Butterfly mimicry rings consisting of Batesian mimics and their toxic models in the Western Ghats, India. Mimicry rings are named after the most common model in each mimicry ring. For all butterfly species, dorsal wing surface is shown on the left and ventral wing surface is shown on the right.

Reflectance Spectrometry

Wing reflectance was measured using an Ocean Optics® Jaz spectrometer with illumination provided by a PX-1 pulsed xenon lamp. Two optical fibers (both outfitted with a collimating lens) were used: the illuminating fiber was positioned at 90° to the wing surface and the collecting fiber was set at 45° to minimize glare. Ambient light was excluded by using a paper-box with black felt, and all spectra were measured relative to a Spectralon® reflectance standard which reflects > 96% of incident light.

For all specimens, I took spectral measurements from both dorsal and ventral wing surfaces. Markings on the forewing and/or hindwing with similar reflectance were categorized as a single colour patch, and the number of repeated readings per colour patch varied from two to eight depending on its extent on the wing. All butterfly species were represented by two dorsal and two ventral colour patches, except for those belonging to the *genutia* mimicry ring, which had only two dorsal colour patches because mimetic convergence was absent on the ventral side, as well as species in the *Delias* mimicry ring, which had one dorsal and three ventral colour patches (refer to Figure 2.3). Spectral readings of different species belonging to the same mimicry ring were measured from corresponding wing regions. For each colour patch, I used the averaged reflectance values between 300 to 700 nm (interpolated to a step width of 1 nm) of each individual for further analysis (see Visual Modeling).

Visual Modeling

Avian colour vision

Insectivorous birds that predate on butterflies are presumably the greatest selective agents and hence intended receivers of butterfly mimicry (Chai, 1996; Pinheiro, 2003, 2011; Langham, 2004, 2006). Avian colour vision is mediated by four classes of single-cone photoreceptors: long-wavelength sensitive (LWS), medium-wavelength sensitive (MWS), short-wavelength sensitive (SWS), and ultraviolet/violet sensitive (UVS/VS) (Hart & Hunt, 2007). The peak sensitivities of LWS, MWS, and SWS photoreceptors are highly conserved across avian taxa, whereas the spectral sensitivity of

UVS/VS photoreceptors peaks either near 370 nm (UVS) or 410 nm (VS) (Hart, 2001a; Hart & Hunt, 2007). As a result, bird species in general fall into two categories: those with UVS and those with VS vision. VS vision is the ancestral state in birds and UVS vision has evolved independently several times (Ödeen & Håstad, 2003, 2013; Carvalho *et al.*, 2007).

Receptor noise model

The receptor noise model (Vorobyev & Osorio, 1998; Vorobyev *et al.*, 2001a), which assumes that discrimination is limited by noise originating in photoreceptors, was used to estimate colour discriminability between butterfly mimics and their models. The model determines the discriminability of any two colours by calculating the perceptual distance between them based on differences in photoreceptor quantum catch and receptor noise (Vorobyev et al. 1998).

To model avian perception, the quantum catch Q_i for each cone type i is first calculated as a function of the photoreceptor spectral sensitivity (S_i), the irradiance spectrum incident on the colour patch (I), and the reflectance spectrum of the patch (R) over the visible spectrum (i.e., 300 to 700 nm):

$$Q_i = \int_{300}^{700} S_i(\lambda) I(\lambda) R(\lambda)\, d\lambda$$

According to Fechner's law, the receptor signal f_i of cone type i is proportional to the logarithm of the quantum catch; the difference in receptor signal Δf_i between two colours A and B is hence given by:

$$\Delta f_i = f_{iA} - f_{iB} = \ln Q_{iA} - \ln Q_{iB} = \ln \frac{Q_{iA}}{Q_{iB}}$$

Receptor noise for each cone type i is defined by the noise-to-signal ratio known as the Weber fraction (ω_i), which is derived from the noise-to-signal ratio of a single receptor (v_i) and the relative number of each receptor type (n_i):

$$\omega_i = \frac{v_i}{\sqrt{n_i}}$$

Colour discrimination using all four cone types in a tetrachromatic visual system is then calculated using the following equation:

$$\Delta S = \sqrt{\frac{\begin{bmatrix} (\omega_1\omega_2)^2(\Delta f_4 - \Delta f_3)^2 + (\omega_1\omega_3)^2(\Delta f_4 - \Delta f_2)^2 + (\omega_1\omega_4)^2(\Delta f_3 - \Delta f_2)^2 + \\ (\omega_2\omega_3)^2(\Delta f_4 - \Delta f_1)^2 + (\omega_2\omega_4)^2(\Delta f_3 - \Delta f_1)^2 + (\omega_3\omega_4)^2(\Delta f_2 - \Delta f_1)^2 \end{bmatrix}}{(\omega_1\omega_2\omega_3)^2 + (\omega_1\omega_2\omega_4)^2 + (\omega_1\omega_3\omega_4)^2 + (\omega_2\omega_3\omega_4)^2}}$$

The colour distance ΔS, given in units of 'just noticeable difference' (jnd), describes the perceptual distance between two spectra, such that the higher the value, the more distinguishable the two colours (Siddiqi, 2004; Cazetta *et al.*, 2009).

Model parameters

Insectivorous birds found in the Western Ghats include drongos (Dicruridae), bee-eaters (Meropidae) and flycatchers (Muscicapidae) (Ali & Ripley, 2002). The avian families Dicruridae and Meropidae have VS vision, whereas the family Muscicapidae has UVS vision (Ödeen *et al.*, 2011; Ödeen & Håstad, 2013); therefore visual modeling was done for both types of avian vision. As the receptor sensitivities of these particular bird species are unknown, the averaged spectral sensitivities (calculated from species with available data) of each visual system were used (see Endler & Mielke 2005; Figure 2.2). In addition, I used the standard illuminant 'D65', which represents standard daylight (Vorobyev *et al.*, 1998), as the irradiance spectrum in quantum catch calculations because birds are most likely to encounter butterflies during the day.

As relative cone abundance is known for only a small handful of bird species (Hart, 2001b), receptor noise was determined using the retinal cone proportions of the blue tit *Cyanistes caeruleus* (1:1.92:2.68:2.7 for SWS1:SWS2:MWS:LWS cones; Hart et al. 2000), which is an arboreal insectivore. Although the blue tit has UVS vision (Ödeen & Håstad, 2013), its cone proportions were used in both sets of modelling (UVS and VS) because there is some evidence to suggest that bird species with similar feeding ecology have similar relative receptor numbers (Hart, 2001b). Lastly, I used a Weber fraction of 0.05 for the most abundant receptor type, following recently published studies (e.g., Avilés 2008; Langmore et al. 2009, 2011; Stoddard & Stevens 2011; Antonov et al. 2012; Delhey et al. 2013). All modelling was performed using the R package *pavo* (Maia *et al.*, 2013).

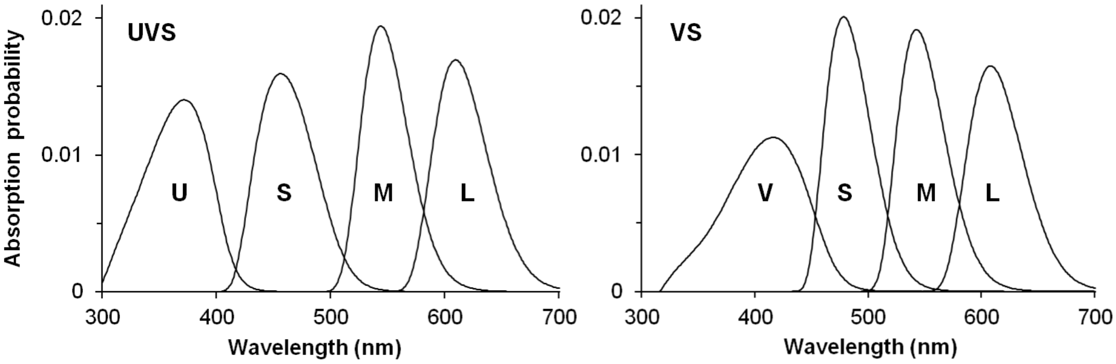

Figure 2.2. Photoreceptor spectral sensitivities of the two types of avian vision (UVS and VS). Letters U, V, S, M, and L refer to the different cone types with peak sensitivity at ultraviolet, violet, short, medium, and long wavelengths, respectively. Taken from Endler & Mielke (2005).

Relative Discriminability

Butterfly colouration exhibits intraspecific phenotypic variation which must be considered when determining whether wing colours of models and mimics are discriminable. To do that, I used the approach given in Håstad et al. (2005) to derive the relative discriminability of two colours. First, I calculated the average interspecific colour distance between the mimic and its model (ΔS_a) and the average intraspecific colour distance among model conspecifics (ΔS_b) from the output of the receptor noise model. The intraspecific distance ΔS_b provides an estimate of wing colour variation shown by the model, and the relative discriminability of the mimic against the model is then given by:

$$D = \frac{\Delta S_a - \Delta S_b}{\sqrt{\Delta S_b}}$$

Mimetic resemblance is thus described by the average colour distance of the mimic to the model relative to the average colour distance among model conspecifics. The mimic is indistinguishable from the model when the colour difference between them is less than or comparable to the colour variation within the model, i.e., when $\Delta S_a \approx \Delta S_b$. Therefore, two colours with $D \leq 0$ are impossible to discriminate, whereas colour pairs with $D < 2$ could be considered as poorly discriminable (Håstad *et al.*, 2005). In general, the lower the D value, the better the mimicry is.

Furthermore, although butterfly models appear to have sexually monomorphic wing colours, they might be sexually dimorphic from an avian visual perspective. To determine whether birds can discriminate between the sexes, I calculated the average intersexual colour distance (ΔS_c) and the average intrasexual colour distance (ΔS_d) for each model species. The relative discriminability of male and female models is then determined as:

$$M = \frac{\Delta S_c - \Delta S_d}{\sqrt{\Delta S_d}}$$

Statistical Analyses

Butterfly models were found to exhibit interspecific variation in intersexual relative discriminability, with some species being sexually monomorphic (i.e., $M \leq 0$ for all colour patches), while others were sexually dimorphic (i.e., $M > 0$ for some or all colour patches) (see Appendix, Table 1). Therefore, I calculated relative discriminability D for mimics with respect to male and female models separately for all colour patches. Also, for sexually monomorphic mimics, I calculated D values for male and female mimics separately (D values were calculated only for females for female-limited mimetic species). As D values were not normally distributed (Shapiro-Wilk test), Wilcoxon rank-sum tests and Wilcoxon signed-rank tests were used for all pairwise comparisons. Statistical analyses were performed in R (R Core Team, 2013). Values are presented as mean±SD.

RESULTS

Relative Discriminability

A total of more than 3,000 spectral readings were obtained from over 200 specimens (Figure 2.3). Approximately 16,300 pairwise comparisons of spectra were made to calculate colour distance and relative discriminability between mimics and models. The distributions of relative discriminability values were positively skewed, with values of $0 < D < 1$ being the most common, and ~ 60% of all colour pairs were barely discriminable (i.e., $D < 2$) from an avian visual perspective (Figure 2.4; D values for each mimic-model pair are given in Appendix, Tables 2 to 6).

Comparisons of Mimetic Resemblance

Females of sexually monomorphic mimics had significantly lower D values than their male counterparts on both wing surfaces, indicating that females had better mimetic resemblance than males from an avian UVS (Wilcoxon signed-rank test: dorsal [♂ = 3.62±3.02 vs. ♀ = 2.60±2.67], $V = 55$, $p < 0.001$; ventral [♂ = 2.34±2.78 vs. ♀ = 1.84±2.17], $V = 190$, $p < 0.01$; Figure 2.5A) and VS (Wilcoxon signed-rank test: dorsal [♂ = 3.69±3.02 vs. ♀ = 2.36±2.59], $V = 14$, $p < 0.001$; ventral [♂ = 2.51±3.01 vs. ♀ = 1.71±2.34], $V = 119$, $p < 0.001$; Figure 2.5B) visual perspective.

Female-limited mimics had similar D values as females of sexually monomorphic mimics on both wing surfaces, suggesting that mimetic resemblance in female-limited mimics and sexually monomorphic females were comparable from an avian UVS (Wilcoxon rank-sum test: dorsal [♀-limited mimics = 3.10±3.36 vs. monomorphic ♀ = 2.60±2.67], $W = 1194$, $p = 0.43$; ventral [♀-limited mimics = 2.44±3.40 vs. monomorphic ♀ = 1.84±2.17], $W = 954$, $p = 0.72$; Figure 2.5A) and VS (Wilcoxon rank-sum test: dorsal [♀-limited mimics = 2.14±2.85 vs. monomorphic ♀ = 2.36±2.59], $W = 1041$, $p = 0.73$; ventral [♀-limited mimics = 2.60±3.90 vs. monomorphic ♀ = 1.71±2.34], $W = 1052$, $p = 0.23$; Figure 2.5B) visual perspective.

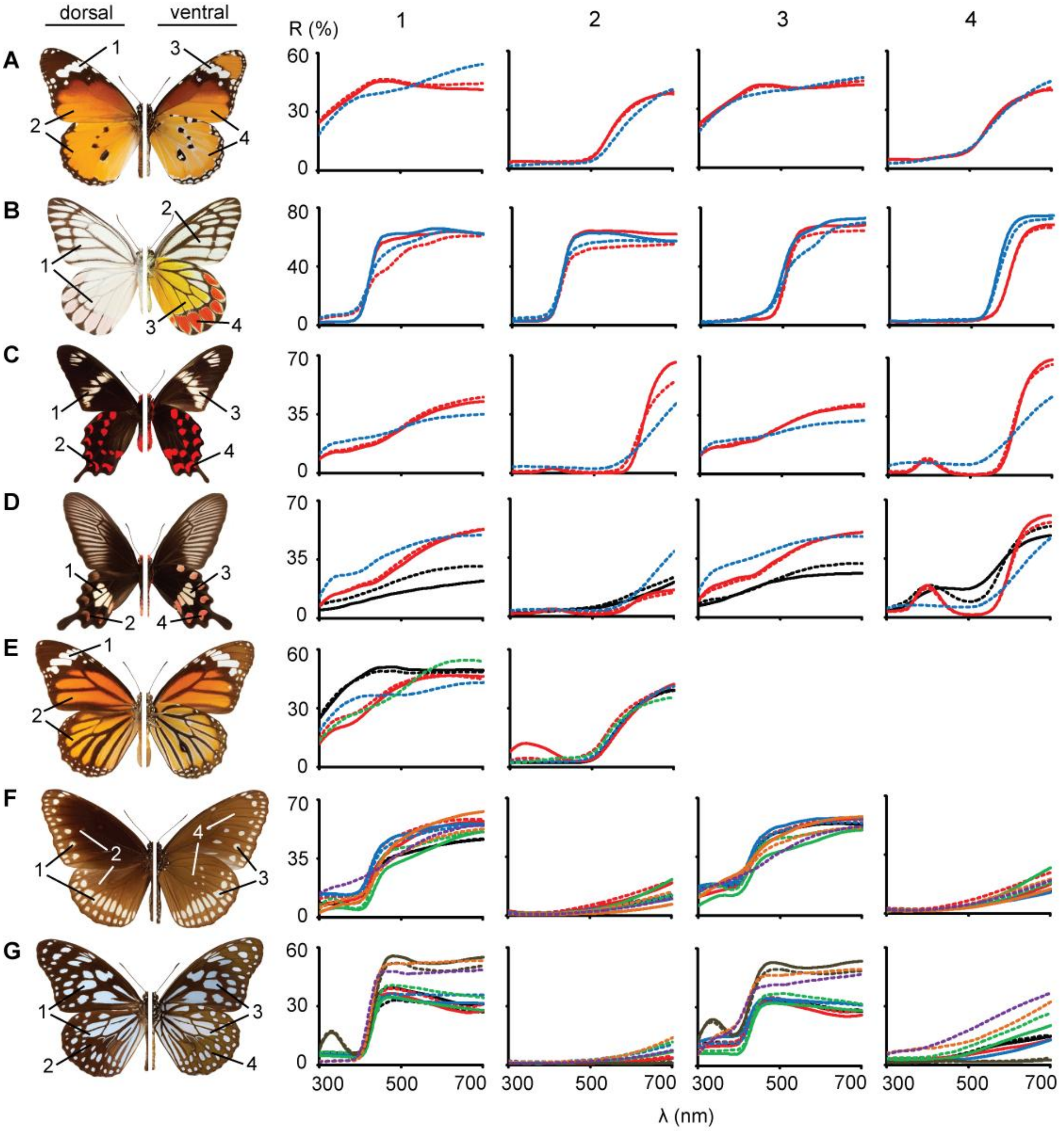

Figure 2.3. Reflectance spectra of butterfly models and mimics in the Western Ghats, India. Rows A to G represent different mimicry rings. One butterfly species from each mimicry ring is shown to indicate wing colour patches (labelled 1 to 4) where spectral readings were taken; wing reflectance of co-models (if any) and mimic(s) were measured from corresponding regions. Repeated measurements (ranging from two to eight) were taken for each colour patch (not shown). Averaged reflectance spectra of males and females of each species are depicted by solid and dashed lines respectively. (A) *chrysippus* mimicry ring: *Danaus c. chrysippus* (red); *Hypolimnas misippus* (blue). (B) *Delias* mimicry ring: *Delias eucharis* (red); *Prioneris sita* (blue). (C) *hector* mimicry ring: *Pachliopta hector* (red); *Papilio polytes romulus* f. *romulus* (blue). (D) *aristolochiae* mimicry ring: *Pachliopta pandiyana* (black); *Pachliopta a. aristolochiae* (red); *Papilio polytes romulus* f. *stichius* (blue). (E) *genutia* mimicry ring: *Danaus g. genutia* (black); *Cethosia mahratta* (red); *Elymnias caudata* (blue); *Argynnis hybrida* (green). (F) *Euploea* mimicry ring: *Euploea c. core* (black); *Euploea klugii kollari* (red); *Euploea sylvester coreta* (blue); *Papilio c. clytia* f. *clytia* (green); *Papilio dravidarum* (brown); *Hypolimnas bolina jacintha* (purple). (G) *Tirumala* mimicry ring: *Tirumala limniace exoticus* (black); *Tirumala septentrionis dravidarum* (red); *Parantica a. aglea* (blue); *Parantica nilgiriensis* (green); *Papilio c. clytia* f. *dissimilis* (gray); *Pareronia c. ceylanica* (brown); *Pareronia hippia* (purple).

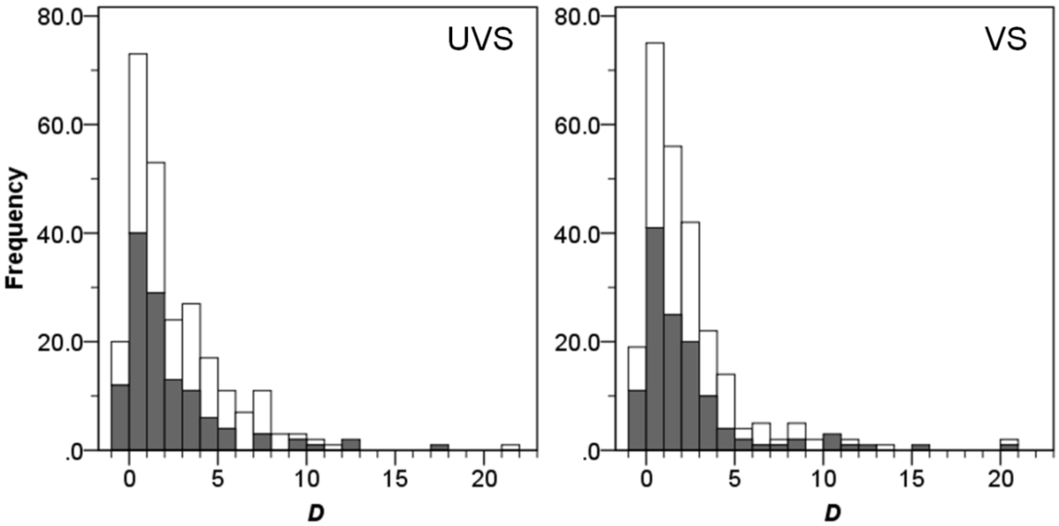

Figure 2.4. Histograms showing the range and frequency of relative discriminability values (i.e., mimetic resemblance) for dorsal (white) and ventral (grey) wing surfaces of butterfly mimics from avian UVS and VS visual perspectives.

Female-limited mimics also had similar *D* values as males of sexually monomorphic mimics in most comparisons, implying that mimetic resemblance in female-limited mimics and sexually monomorphic males were also largely comparable from an avian UVS (Wilcoxon rank-sum test: dorsal [♀-limited mimics = 3.10±3.36 vs. monomorphic ♂ = 3.62±3.02], W = 934, p = 0.25; ventral [♀-limited mimics = 2.44±3.40 vs. monomorphic ♂ = 2.34±2.78], W = 860, p = 0.66; Figure 2.5A) and VS (Wilcoxon rank-sum test: dorsal [♀-limited mimics = 2.14±2.85 vs. monomorphic ♂ = 3.69±3.02], W = 665, $p < 0.01$; ventral [♀-limited mimics = 2.60±3.90 vs. monomorphic ♂ = 2.51±3.01], W = 879, p = 0.78; Figure 2.5B) visual perspective.

Overall, ventral wing surfaces of butterfly mimics had significantly lower *D* values than dorsal wing surfaces, suggesting that mimetic resemblance was better on ventral surfaces as compared to dorsal surfaces (Wilcoxon rank-sum test: avian UVS [dorsal = 3.10±3.11 vs. ventral = 2.23±2.87], W = 9888, $p < 0.01$; avian VS [dorsal = 2.60±2.89 vs. ventral = 2.30±3.22], W = 9391, $p < 0.05$; Figure 2.6A). To determine whether the two avian visual systems differ in their ability to discriminate between models and mimics, *D* values from a UVS perspective were compared with those from a VS perspective. Mimetic resemblance was not significantly different when viewed using either type of avian vision (Wilcoxon signed-rank test: dorsal [UVS = 3.10±3.11 vs. VS = 2.60±2.89], $V = 4929$, $p = 0.22$; ventral [UVS = 2.23±2.87 vs. VS = 2.30±3.22], $V = 3588$, $p = 0.48$; Figure 2.6B).

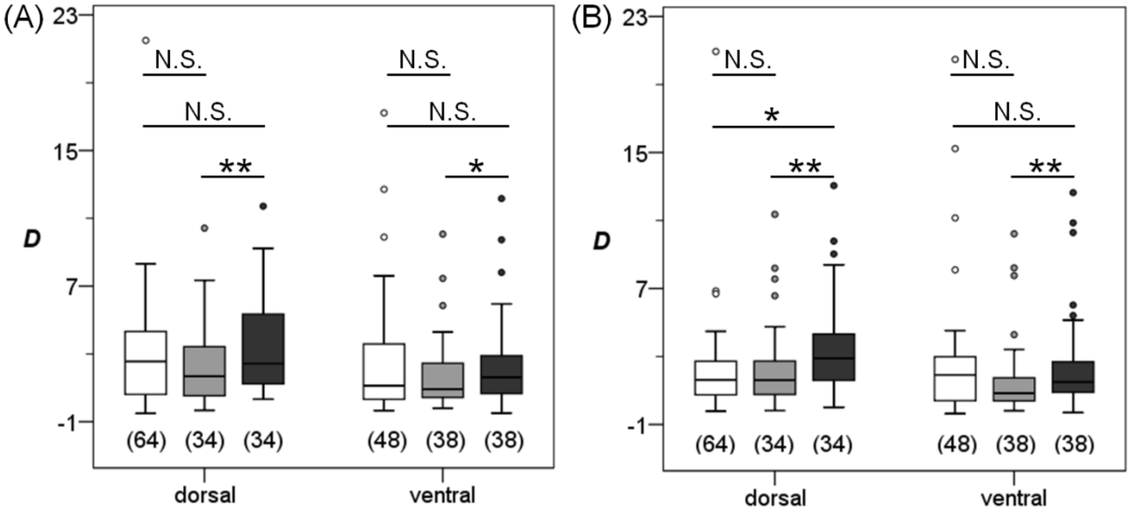

Figure 2.5. Pairwise comparisons of mimetic resemblance between female-limited Batesian mimics (white) and sexually monomorphic Batesian mimics (light grey: females; dark grey: males) from avian UVS (A) and VS (B) visual perspective. Dorsal and ventral wing surfaces are compared separately. Numbers in parentheses indicate sample sizes. N.S. $p > 0.05$; * $p < 0.01$; ** $p < 0.001$.

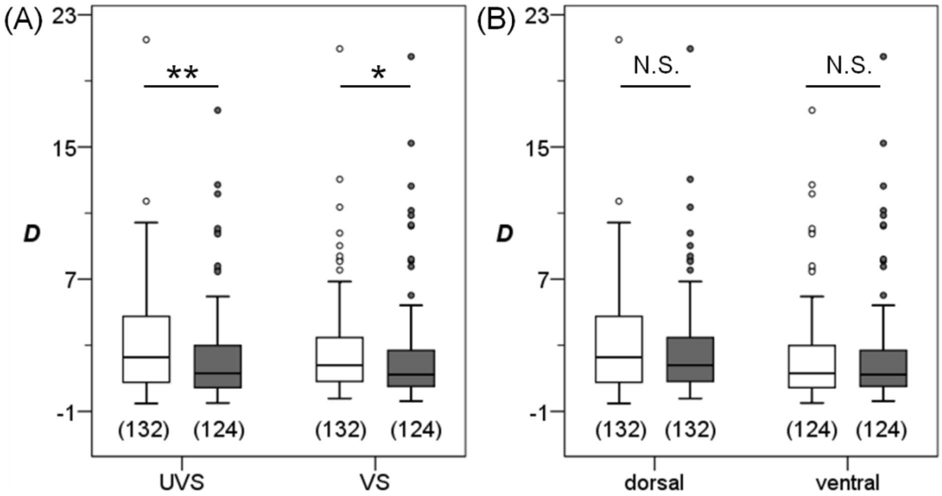

Figure 2.6. (A) Comparisons of mimetic resemblance between dorsal (white) and ventral (grey) wing surfaces of Batesian mimics from avian UVS and VS visual perspective. (B) Comparisons of mimetic resemblance when viewed by avian predators with UVS (white) or VS (grey) vision for dorsal and ventral wing colouration. Numbers in parentheses indicate sample sizes.
N.S. $p > 0.05$; * $p < 0.05$; ** $p < 0.01$.

DISCUSSION

Batesian mimicry, an evolutionary adaptation that results in the reduced predation of palatable species due to their resemblance to toxic models (Bates, 1862), must be studied from the perception of the intended receivers (i.e., predators) for the findings to have ecological relevance (Bennett *et al.*, 1994). Since insectivorous birds are the main predators of butterflies, I analyzed and quantified mimetic resemblance of Western Ghats butterflies using a physiological model of avian colour vision. In agreement with prediction, intraspecific comparisons of mimetic resemblance showed that female butterflies were better Batesian mimics than males. On the other hand, interspecific comparisons revealed that female-limited mimics and sexually monomorphic mimics were equally good mimics. Also contrary to expectation, ventral wing surfaces exhibited greater mimetic resemblance than dorsal wing surfaces. These results suggest that mimetic resemblance in butterflies is influenced by selection pressures acting in a sex- and wing surface-specific manner.

Male vs. Female Mimics

Although males and females of sexually monomorphic mimetic butterflies appear highly similar to the human eye, avian visual modelling revealed that wing colours of the two sexes were not equally discriminable from those of models, with females being better Batesian mimics than their male counterparts. This may be a general pattern across different mimicry types: among *Heliconius* and *Melinaea* butterflies, females are better Müllerian mimics than males (Llaurens *et al.*, 2014). These findings suggest that female butterflies are under stronger natural selection for mimicry. Evidence from several studies indicate that female butterflies may face greater predation risk than males (Bowers *et al.*, 1985; Kingsolver, 1995a, 1995b; Ohsaki, 1995, 2005; Ide, 2006), possibly because females are potentially vulnerable to predators during oviposition at larval host plants, and their heavy egg-loads may also impair flight performance to result in a less effective escape flight (Srygley & Chai, 1990; Srygley & Dudley, 1993; Almbro & Kullberg, 2012). Female-biased predation has also been observed in crickets (Gwynne & Dodson, 1983; O'Neill & O'Neill, 2003; Ercit, 2014), with a recent study demonstrating that egg-loads impede females' ability to escape predation by reducing jumping capacity (Ercit *et al.*, 2014). Thus, differential predation risk between the sexes could lead to the evolution of better mimetic resemblance in females of sexually monomorphic mimics.

A non-mutually exclusive alternative hypothesis to explain the higher mimetic resemblance of female butterflies is that male wing colouration might be under stronger sexual selection, which may act antagonistically to natural selection. Females of several butterfly species show a mating preference for males with brighter and/or more saturated colours (Robertson & Monteiro, 2005; Davis *et al.*, 2007; Kemp, 2007, 2008b; Papke *et al.*, 2007; Morehouse & Rutowski, 2010; Rutowski & Rajyaguru, 2012). However, exaggerated traits which make males more attractive to females may also cause them to become more vulnerable to predation; for instance, Morehouse & Rutowski (2010) found that male cabbage white butterflies which are preferred by females potentially face greater avian predation risk due to their higher conspicuousness. Besides female mate choice, male-male competition could also act antagonistically to natural selection for mimicry. In the butterfly *Papilio polyxenes*, males are less effective dorsal mimics of the toxic *Battus philenor* than females (Codella & Lederhouse, 1989), and males with the natural phenotype were more successful in establishing mating territories than were males altered to have female-like mimetic colouration (Lederhouse & Scriber, 1996). Therefore, sexual selection acting on mimetic male butterflies may cause male colouration to diverge away from the optimal phenotype as predicted under natural selection alone, resulting in lower mimetic resemblance as compared to females.

Female-limited vs. Sexually Monomorphic Mimics

Sexual dimorphism is the evolutionary outcome of sexually antagonistic or sex-specific selection (Cox & Calsbeek, 2009). However, sexually dimorphic traits may still deviate from their sex-specific fitness optimum due to positive intersexual genetic correlations that constrain the independent evolution of the two sexes (Lande, 1987; Price & Burley, 1994; Merila *et al.*, 1998; Badyaev & Martin, 2000; Poissant *et al.*, 2009). For butterflies exhibiting female-limited Batesian mimicry, the evolution of better mimetic resemblance in females may be hampered by genetic correlations between mimetic females and non-mimetic males. Contrary to prediction, I found that female-limited mimics and sexually monomorphic mimics showed comparable mimetic resemblance. This suggests that intersexual genetic correlations did not constrain natural selection for mimicry in female-limited mimics, to the extent that mimetic resemblance of females could evolve to a similar degree as that shown by sexually monomorphic mimics. The small number of gene loci involved in butterfly wing patterning probably also contributes to the independent wing colour evolution of the sexes in female-limited mimetic species (Nijhout, 1994, 2003; Beldade & Brakefield, 2002). Currently, the exact genes involved in wing patterns of female-limited mimetic species are largely unknown (but see Kunte *et al.*, 2014; Nishikawa *et al.*, 2015), and the mechanisms used to overcome or reduce intersexual genetic correlations for these genes remain to be studied (Rhen, 2000; Day & Bonduriansky, 2004; Fairbairn & Roff, 2006).

Dorsal vs. Ventral Wing Surfaces

Dorsal wing patterns of butterflies are exposed during flight and are readily visible to their aerially-hunting avian predators, so dorsal wing surfaces were predicted to show greater mimetic resemblance. Contrary to expectation, I found that ventral wing surfaces of Batesian mimics exhibited better resemblance to their models. As ventral wing surfaces are exposed when butterflies close their wings at rest, this suggests that butterflies may experience greater predation risk when they are resting rather than flying. This finding could also be due to differential natural and sexual selection acting on the two wing surfaces. Males of the butterfly *Papilio polyxenes* are non-mimetic on the dorsal wing surface due to intrasexual selection, whereas the ventral wing surface closely resembles that of the model (Codella & Lederhouse, 1989; Lederhouse & Scriber, 1996). Furthermore, in several species of non-mimetic butterflies, dorsal characters or colours are involved in conspecific signalling while ventral colouration plays a role in predator avoidance (Breuker & Brakefield, 2002; Balint *et al.*, 2009; Oliver *et al.*, 2009). Similarly, the ventral iridescent blue of the aposematic butterfly *Battus philenor* functions as a warning signal of its toxicity, while dorsal blue iridescence acts as a sexual signal (Codella & Lederhouse, 1990; Rutowski *et al.*, 2010). Together, these findings suggest that natural selection by avian predators acts more strongly on ventral colouration, whereas dorsal colouration may be subject to greater sexual selection. Therefore, stronger natural selection for Batesian mimicry on the ventral wing surface and/or antagonistic sexual selection on dorsal colouration may explain the wing surface-specific difference in mimetic resemblance found in this study.

Avian UVS vs. VS Vision

Butterfly mimetic resemblance was analyzed for both types of avian vision (i.e., UVS and VS) because insectivorous birds of the Western Ghats fall into both categories (Ödeen *et al.*, 2011; Ödeen & Håstad, 2013). Comparisons of mimetic resemblance derived from the UVS and VS perspective respectively showed that mimetic resemblance was similar between the two types of avian vision. There is some evidence to suggest that the UVS visual system is better than the VS system at discriminating or detecting colours (Vorobyev *et al.*, 1998; Håstad *et al.*, 2005; Schaefer *et al.*, 2007; Avilés & Soler, 2009; Morehouse & Rutowski, 2010; Delhey *et al.*, 2013), possibly due to the smaller spectral overlap between the UVS and SWS photoreceptors. However, the relative advantage of the UVS system appears to be restricted to narrow parts of the spectrum where sensitivities of the two visual systems are different (e.g., 410 to 425 nm or 470 to 490 nm; Schaefer *et al.*, 2007; 300 to 500 nm; Ödeen *et al.*, 2012; Delhey *et al.*, 2013). The majority of butterfly wing spectra collected in this study were not particularly rich in short wavelengths (< 500 nm), with most of the spectra showing an even reflectance from 400 to 700 nm (e.g., white, brown) or mainly at longer wavelengths (e.g., orange, red). Since the two avian visual systems are equally sensitive to middle and long wavelengths, it is perhaps not surprising that no difference in mimetic resemblance was detected (see also Langmore *et al.*, 2011; Honza *et al.*, 2014). Alternatively, it could also be because the wing colouration of mimetic butterflies has evolved under similar predation pressure imposed by both groups of birds.

Future Studies

Wing colour pattern

In the present study, the resemblance between Batesian mimics and their respective models was quantified based on wing colouration. Although colour is often the most salient and effective cue in aposematism and mimicry (Terhune, 1977; Gamberale-Stille & Guilford, 2003; Exnerová *et al.*, 2006; Aronsson & Gamberale-Stille, 2008; Svádová *et al.*, 2009; Finkbeiner *et al.*, 2014), pattern (i.e., the spatial arrangement of markings) has also been shown to be involved in avian learning of aposematism (Terhune, 1977; Forsman & Merilaita, 1999; Aronsson & Gamberale-Stille, 2012), suggesting that it could play a role in mimicry as well. Stoddard (2012) examined the wing patterns of Neotropical butterflies belonging to five different mimicry rings, and found that wing patterns closely cluster according to mimicry ring, indicating that wing patterns within a mimicry ring are highly repeatable and distinct from those of other rings. Similarly, a study by Taylor *et al.* (2013) demonstrated that the highest similarity values for a given wing pattern were shown by co-mimetic pairs of *Heliconius* and *Melinaea* butterflies. These findings lend support to the hypothesis that both colour and pattern are important in butterfly mimicry (e.g., Finkbeiner *et al.*, 2014). To test this hypothesis in Western Ghats butterflies, quantification of wing patterns of mimics and their models could be done using granularity analysis (Stoddard, 2012) or distance transform (Taylor *et al.*, 2013) to provide an objective assessment of mimetic resemblance based on wing pattern.

Locomotor mimicry

Other than colouration and pattern, butterfly locomotion is also an important visual signal that affects the feeding responses of avian predators (Chai, 1986). Palatable butterflies generally have a fast and erratic flight to make it difficult for predators to predict the flight path, whereas toxic butterflies typically fly slowly and regularly to enhance the conspicuousness of their aposematic colouration (Chai & Srygley, 1990; Srygley & Chai, 1990; Chai, 1996; Pinheiro, 1996). Due to the distinct behavioural difference between these two groups of butterflies, palatable butterflies which are mimetic may experience predator pressure to evolve a similar flight pattern as their toxic models. Evidence of locomotor mimicry in mimetic butterflies has been found (Srygley & Ellington, 1999; Srygley, 2004; Kitamura & Imafuku, 2010, 2015). Furthermore, as a result of locomotor mimicry, flight in mimetic butterflies has been shown to be more costly than in their models or in closely related non-mimetic butterflies (Srygley, 2004). The evolution of flight mimicry in Batesian mimics despite its energetic cost strongly implies that avian predators are sensitive to this particular aspect of prey appearance. Since flight pattern is likely to form an essential part of the overall mimetic signal, future research could examine whether Western Ghats butterfly mimics and their models display such behavioural convergence within individual mimicry rings. In addition, polymorphic mimics with both mimetic and non-mimetic forms (e.g., *Papilio polytes*) or with models belonging to different mimicry rings (e.g., *Papilio clytia*) would be ideal candidates for the investigation of locomotor mimicry in butterflies (see Kitamura & Imafuku, 2010).

Behavioural studies

Despite the application of a physiological model of avian colour vision, differences in butterfly mimetic resemblance detected in this study may not necessarily have ecological significance (for example, males and females of sexually monomorphic mimics might be equally protected even though females displayed higher mimetic resemblance). This is because the output of visual modelling does not consider higher cognitive mechanisms such as colour generalization, which birds are known to possess (Exnerová *et al.*, 2006; Ham *et al.*, 2006; Svádová *et al.*, 2009; Sandre *et al.*, 2010; Pegram & Rutowski, 2014). Therefore, behavioural studies of avian predation on mimetic butterflies are required to assess the true effectiveness of mimicry; such experimental data are currently available mainly for Neotropical butterflies (Brower, 1958; Platt *et al.*, 1971; Chai, 1986; Codella & Lederhouse, 1989; Ritland, 1991, 1998; Prudic *et al.*, 2002; Pinheiro, 2003; Long *et al.*, 2014). Furthermore, prey generalization by predators is dynamic and depends on numerous factors, including the availability of alternative prey (Kokko *et al.*, 2003a; Sherratt, 2003; Lindstrom *et al.*, 2004; Honma *et al.*, 2008) as well as model abundance and toxicity (Sherratt, 2002; Darst & Cummings, 2006; Harper & Pfennig, 2007). Generally, when alternative palatable prey is readily available or when the model is abundant and highly toxic, selection for mimetic perfection is relaxed such that both good and poor mimics receive similar levels of protection. Due to the complexity of the foraging decisions of predators, behavioural studies to evaluate the effectiveness of butterfly mimicry should preferably be conducted under more natural conditions (e.g., Chai, 1986; Pinheiro, 2003) for the findings to have ecological relevance.

CHAPTER 3

SEXUAL DICHROMATISM AND BATESIAN MIMICRY IN SWALLOWTAIL BUTTERFLIES

ABSTRACT

Sexual dichromatism is a widespread form of sexual dimorphism among animals. Although it is often treated as an indicator of sexual selection, natural selection acting in a sex-specific manner can also result in the evolution of sexual dichromatism. Several species of swallowtail butterflies (genus *Papilio*) exhibit female-limited Batesian mimicry, whereby females are mimetic and males retain non-mimetic wing colour patterns. Here I investigated the relationship between sexual dichromatism and Batesian mimicry in Mormon swallowtail butterflies (subgenus *Menelaides*). First, I showed that sexual dichromatism in this subgenus was closely associated with the presence of female-limited mimicry, indicating that natural selection is the main driver of sexual dichromatism in these butterflies. Next, using reflectance spectrometry and visual modelling of avian perception, I found that mimetic butterflies displayed adaptive wing colour changes that were not apparent to the human observer. Lastly, I demonstrated that butterfly species with sexually monomorphic wing patterns were sexually dimorphic in terms of brightness from the visual perspectives of both butterfly conspecifics and avian predators. These results emphasize the importance of quantifying animal colouration objectively to avoid the biases of human vision, and highlight the significance of analyzing colour measurements using approaches that take into consideration the sensory capabilities of the intended receivers. I discussed the evolutionary implications of these findings and suggested promising avenues for future work.

INTRODUCTION

Sexual dichromatism is a widespread form of sexual dimorphism in which males and females of the same species differ in colouration. It has been documented in many major animal groups, including birds (Badyaev & Hill, 2003), fishes (Kodric-Brown, 1998), mammals (Caro, 2005), lizards (Stuart–Fox & Ord, 2004; Lanuza *et al.*, 2013), frogs (Bell & Zamudio, 2012), and butterflies (Allen *et al.*, 2011). Like other types of sexual dimorphism, sexual dichromatism has often been treated as an indicator of the presence and intensity of sexual selection (Andersson, 1994). However, natural selection such as predation risk may also cause the evolution of sexual dichromatism by favoring protective colouration in one sex but not the other (Götmark, 1993; Martin & Badyaev, 1996; Götmark *et al.*, 1997). As a result of natural and sexual selection acting differentially on the sexes, males generally tend to be the more conspicuous sex whereas females tend to have more cryptic coloration (but see Heinsohn et al. 2005; Tobias et al. 2012).

Swallowtail butterflies (genus *Papilio*) provide a unique example of how natural selection could drive the evolution of sexual dichromatism. With over 200 species distributed worldwide, these charismatic butterflies exhibit spectacular diversity in wing colour patterns (Figure 3.1). Many *Papilio* species are Batesian mimics, i.e., they gain protection from predators by resembling unpalatable species known as models (Bates, 1862). Furthermore, in several species mimicry is limited to the female sex, such that females are mimetic whereas males are non-mimetic (Kunte, 2009a). The evolution of female-limited mimicry has been attributed to the higher predation risk faced by female butterflies (Kingsolver, 1995a, 1995b; Ohsaki, 1995, 2005; Ide, 2006), which potentially have a less effective escape flight than males due to their heavy egg-loads (Srygley & Chai, 1990; Srygley & Dudley, 1993). In addition, stabilizing sexual selection on male wing colouration may also play a role (Krebs & West, 1988; Lederhouse & Scriber, 1996). Since females show mimetic wing patterns while males retain non-mimetic ancestral colouration, female-limited mimetic species have sexually dimorphic wing colour patterns. A phylogenetic analysis of *Papilio* species illustrated the close association of sexual dichromatism and female-limited Batesian mimicry in this group of butterflies, with the origin of the latter driving the evolution of the former (Kunte, 2008).

In addition to showing remarkable Batesian mimicry, it has long been noted that many *Papilio* species possess wing colour patches which emit a blue-green fluorescence under ultraviolet illumination (Cockayne, 1924). This is due to the presence of a fluorescent pigment known as papiliochrome II, which is a combination of N-β-alanyldopamine and kynurenine (Umebachi, 1977, 1985). Among *Papilio* butterflies, both mimetic and non-mimetic species/sexes frequently have white (as perceived by humans) patches on their hindwings; however, only white patches of non-mimetic species or sexes were observed to fluoresce (personal observations). This suggests, firstly, that papiliochrome II may not be present in the white patches of mimetic species/sexes. Secondly, the absence of fluorescence in mimetic *Papilio* butterflies implies that this may be an adaptive response to natural selection for Batesian mimicry. If the loss of fluorescence in mimetic butterflies is adaptive, the associated change in wing colouration should result in them having a closer resemblance to their models, as compared to non-mimetic butterflies which retain the fluorescent signal. Since insectivorous birds that predate on butterflies are presumably the greatest selective agents and hence intended receivers of butterfly mimicry (Chai, 1996; Pinheiro, 2003, 2011; Langham, 2004, 2006), this must be assessed using avian perception, which differs considerably from human colour vision (reviewed by Cuthill et al. 2000).

Even among butterfly species with sexually monomorphic wing colour patterns, males are often observed to have brighter or more saturated colouration, while females are described as having duller colours (Darwin, 1871; Silberglied, 1984; Rutowski, 1997). Although this trend has been frequently observed across diverse butterfly species, it has rarely been quantified objectively and studied from the relevant visual perspective of the intended receivers. Butterfly wing colours play an important role in sexual signaling (Robertson & Monteiro, 2005; Kemp, 2007, 2008b; Papke *et al.*, 2007; Morehouse & Rutowski, 2010; Merrill *et al.*, 2011; Rutowski & Rajyaguru, 2012), and hence they should be appropriately assessed using the perception of conspecifics. Behavioural evidence indicates that *Papilio* butterflies have tetrachromatic colour vision and are sensitive to wavelengths ranging from 300 to 700 nm (Kinoshita *et al.*, 1999; Kinoshita & Arikawa, 2000; Kelber *et al.*, 2001). Due to differences in visual capabilities, butterflies may exhibit sexual colour differences which are not apparent to the human observer. Evidence from several studies suggest that human perception is unlikely to provide a reliable estimate of sexual dichromatism in birds (Hunt *et al.*, 1998; Cuthill *et al.*, 1999; Mahler & Kempenaers, 2002; Eaton, 2005; Tubaro *et al.*, 2005). In addition, as brighter and more saturated wing colours may also increase conspicuousness to predators (e.g., Morehouse & Rutowski, 2010), the perception of avian predators should also be considered.

In this study, I investigated sexual dichromatism and Batesian mimicry in Mormon swallowtail butterflies (subgenus *Menelaides*) with three objectives. With approximately 60 species distributed over the Indo-Australian region, Mormon swallowtails make up the largest subgenus of *Papilio*, and include iconic mimetic species such as *Papilio memnon* and *P. polytes*. My first objective was to determine whether sexual dichromatism in these butterflies is closely associated with the presence of female-limited Batesian mimicry. Second, I characterized, using reflectance spectrometry, the white patches found on hindwings of mimetic and non-mimetic *Papilio* butterflies, as well as those of toxic butterfly species (potential models for Batesian mimics). Applying a visual model of avian perception, I evaluated whether mimetic butterflies show adaptive colour changes by comparing the wing colouration of mimetic and non-mimetic butterflies with those of toxic species. Lastly, I examined the presence of sexual dichromatism in butterfly species with sexually monomorphic wing colour patterns from both a butterfly (i.e., conspecific) and an avian (i.e., predator) visual perspective. Specifically, I tested whether males have brighter white patches and darker black regions on their hindwings than females, as this has often been observed to be the case. Evolutionary significance and implications of the findings were discussed, and I suggested promising avenues for future work.

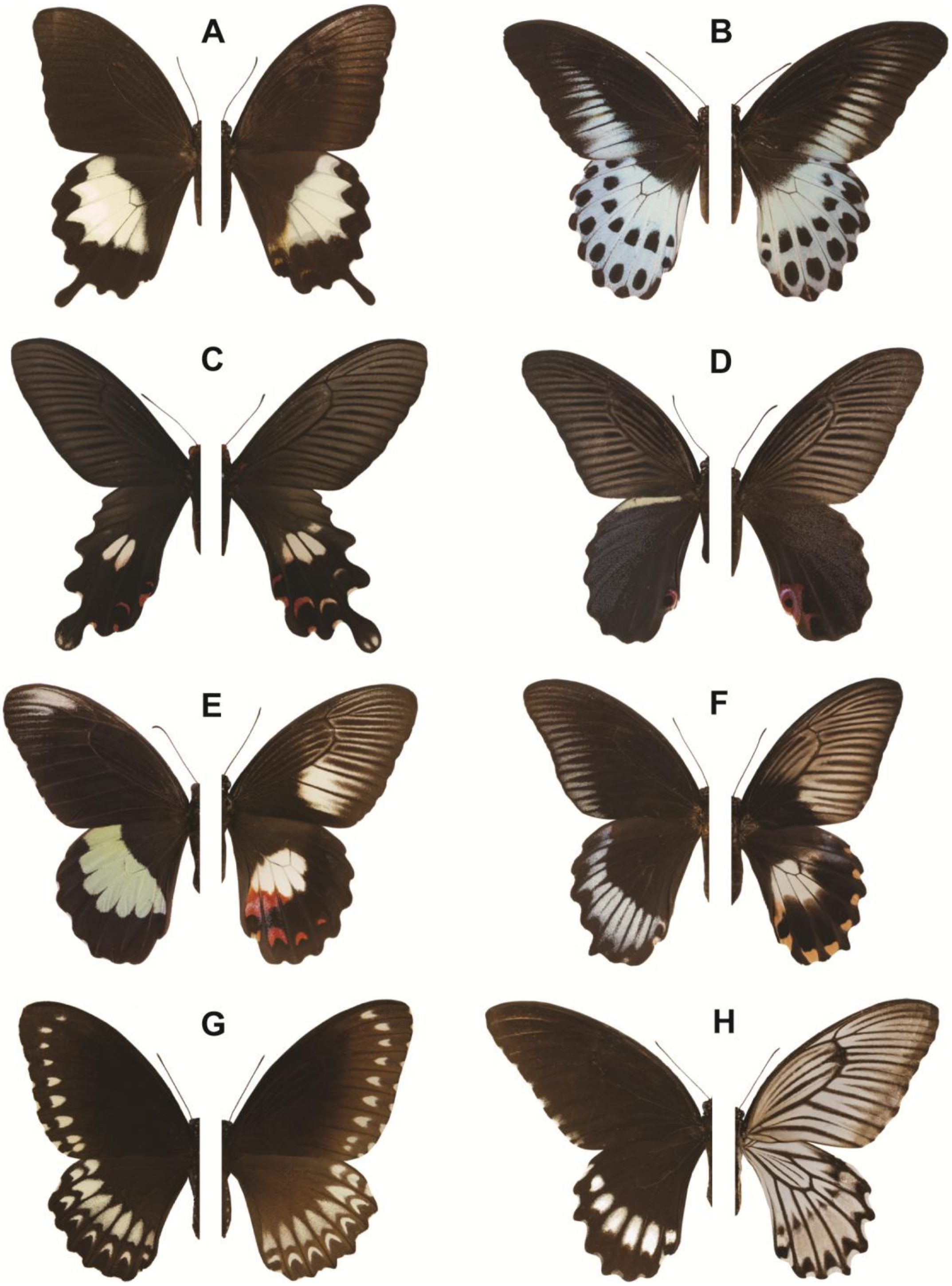

Figure 3.1. *Papilio* butterflies exhibit diverse wing colour patterns. (A) *P. albinus;* (B) *P. polymnestor;* (C) *P. bootes;* (D) *P. protenor;* (E) *P. ambrax;* (F) *P. deiphontes;* (G) *P. dravidarum;* (H) *P. jordani*. For all species, only dorsal wing surfaces are shown. Left and right images represent males and females, respectively.

METHODS

Classification of Sexual Dichromatism and Mimicry

First, butterfly species were classified as sexually dichromatic when wing colour patterns of males and females show unsubtle differences. Next, species/sexes were considered to be Batesian mimics when their wing colour patterns closely resemble those of toxic butterfly species which occur in sympatry (D'Abrera, 1982; Braby, 2004). These sympatric toxic butterfly species belong to the tribe Troidini (e.g., *Pachliopta* spp.) or Danaini (e.g., *Euploea* spp.) (Figure 3.2). Their larvae feed on plants that contain toxic secondary chemical compounds and sequester these compounds for chemical defense, rendering both larvae and adults unpalatable to predators (Ackery & Vane-Wright, 1984; Boppré, 1986; Nishida & Fukami, 1989; Klitzke & Brown, 2000; Sime *et al.*, 2000; Wu *et al.*, 2000). Due to their unpalatability, butterfly species from these tribes often serve as models for Batesian mimics (Brower, 1958; Smith, 1973; Codella & Lederhouse, 1989; Uésugi, 1995, 1996; Gordon *et al.*, 2010). Lastly, mimicry was categorized as sexually monomorphic (i.e., both sexes mimic the same model), female-limited (i.e., females are mimetic and males are non-mimetic), and sexually dimorphic (i.e., females and males mimic different models).

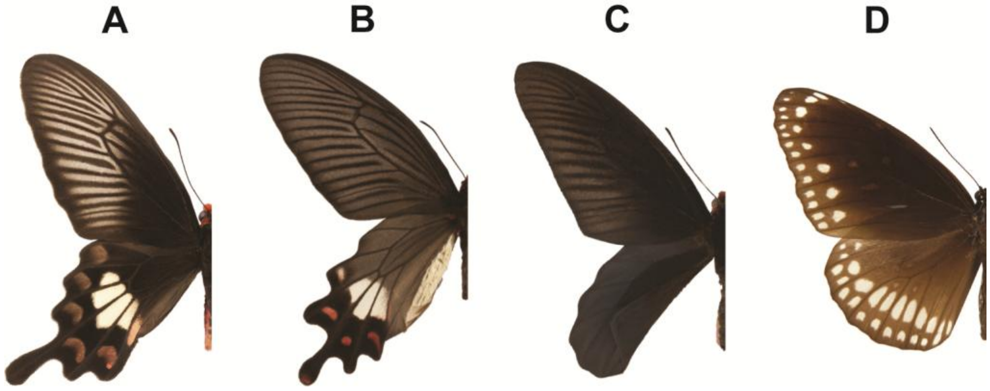

Figure 3.2. Toxic butterfly models of *Papilio* Batesian mimics. (A) *Pachliopta aristolochiae;* (B) *Byasa latreillei;* (C) *Atrophaneura varuna;* (D) *Euploea core.* For all species, only dorsal wing surfaces are shown.

Reflectance Spectrometry

Reflectance spectra were obtained from butterfly specimens deposited in the Natural History Museum (London, United Kingdom) and the Research Collections facility at the National Centre for Biological Sciences (Bangalore, India). For each butterfly species, wing colouration of one male and one female were measured using the best specimens available (i.e., with no or minimal wing damage). Wing reflectance was measured using an Ocean Optics® Jaz spectrometer with illumination provided by a PX-1 pulsed xenon lamp. Two optical fibers (both outfitted with a collimating lens) were used: the illuminating fiber was positioned at 90° to the wing surface and the collecting fiber was set at 45° to minimize glare. Ambient light was excluded by using a paper-box with black felt, and all spectra were measured relative to a Spectralon® reflectance standard which reflects > 96% of incident light. For each specimen, spectral readings were taken from white patches (if any) and black/brown patches found on the dorsal and ventral hindwings. Repeated measurements (ranging from two to four) were collected for each colour patch. Averaged reflectance values between 300 to 700 nm (interpolated to a step width of 1 nm) were used for further analysis.

Visual Modeling

Avian colour vision

Four classes of single-cone photoreceptors are involved in avian colour vision: long-wavelength sensitive (LWS), medium-wavelength sensitive (MWS), short-wavelength sensitive (SWS), and ultraviolet/violet sensitive (UVS/VS) (Hart & Hunt, 2007). The peak sensitivities of LWS, MWS, and SWS cones are highly conserved across avian taxa, whereas the spectral sensitivity of UVS/VS photoreceptors peaks either near 370 nm (UVS) or 410 nm (VS) (Hart, 2001a). As a result, bird species can be categorized into two groups: those with VS vision, which is the ancestral state, and those with UVS vision, which has evolved independently several times (Ödeen & Håstad, 2003, 2013; Carvalho *et al.*, 2007).

Butterfly colour vision

The butterfly compound eye consists of several thousands of ommatidia which contain photoreceptor cells (Stavenga & Arikawa, 2006; Frentiu, 2010). Among *Papilio* butterflies, the visual system of *P. xuthus* is most well-studied (reviewed by Kinoshita & Arikawa 2014). Six classes of photoreceptors have been identified in *P. xuthus*, namely the ultraviolet (UV), violet (V), blue (B), green (G), red (R), and broadband (BB) receptors (Arikawa, 2003). The UV, V, B, G, and R receptors have distinct peak sensitivities at 360, 400, 460, 520, and 600 nm respectively, whereas the BB receptor has a very wide spectral sensitivity that spans from about 450 to 650 nm (Arikawa et al. 2003; Figure 3.3). In addition, the B and G receptors each have two subtypes: the spectral sensitivities of B receptors can be narrow (nB) or wide (wB) (Kinoshita *et al.*, 2006), while those of G receptors can be double- (dG) or single-peaked (sG) (Arikawa *et al.*, 1999a). These photoreceptors are distributed in fixed combinations to form three types of ommatidia: type I ommatidia contain UV, nB, dG and R receptors; type II ommatidia have V, sG and BB receptors; and type III ommatidia consists of wB and dG receptors (Arikawa 2003; Figure 3.3). Despite having six photoreceptor classes, the colour vision of *P. xuthus* is tetrachromatic, involving the UV, B, G, and R receptors in type I and III ommatidia (Koshitaka *et al.*, 2008). Type II ommatidia do not seem to play a role in colour perception (Koshitaka *et al.*, 2008). In addition, brightness perception appears to be mediated by the BB receptor (Koshitaka *et al.*, 2011).

Colour space and volume overlap

For an animal with tetrachromatic colour vision, any perceived colour can be mapped as a point in a tetrahedral space, with its position in the tetrahedron determined by the relative stimulation of the four photoreceptor types (Goldsmith, 1990; Endler & Mielke, 2005; Stoddard & Prum, 2008). The four vertices of the tetrahedron correspond to the four different photoreceptors, i.e., UVS/VS, SWS, MWS, and LWS cones for avian colour vision, and UV, B, G, and R receptors for butterfly colour vision. In this three-dimensional space, the achromatic point of equal receptor stimulation (i.e., white, black or grey) is placed at the origin (0,0,0) and any colour can be represented by its X, Y, and Z Cartesian coordinates.

To map butterfly wing colouration in avian or butterfly colour space, first, the photon catch Q_i for each cone type i was calculated as a function of the photoreceptor spectral sensitivity (S_i), the irradiance spectrum incident on the colour patch (I), and the reflectance spectrum of the patch (R) over the visible spectrum (i.e., 300 to 700 nm):

$$Q_i = \int_{300}^{700} S_i(\lambda) I(\lambda) R(\lambda)\, d\lambda$$

For avian colour space, I used the averaged spectral sensitivities of each avian visual system (i.e., UVS and VS; given in Endler & Mielke, 2005) to represent diverse avian predators. For butterfly colour space, I calculated the spectral sensitivities of *Papilio xuthus* photoreceptors (i.e., UV, nB, dG and R) using the equation and parameters provided in Koshitaka et al. (2008). The standard illuminant ‘D65’, which represents standard daylight, was used as the irradiance spectrum.

Next, the von Kries transformation (Vorobyev *et al.*, 2001b), which accounts for light adaptation, was applied to achieve colour constancy:

$$q_i = \frac{\int_{300}^{700} S_i(\lambda) I(\lambda) R(\lambda)\, d\lambda}{\int_{300}^{700} S_i(\lambda) I(\lambda)\, d\lambda}$$

As colour perception depends on the relative (and not absolute) stimulation of photoreceptors, q_i values were then normalized to sum to 1 to yield relative values: {*u/v, s, m, l*} for avian colour vision and {*u, b, g, r*} for butterfly colour vision. This step also removes the achromatic dimension (i.e., brightness), which is thought to be processed independently of colour (Osorio *et al.*, 1999a; Osorio & Vorobyev, 2005; Koshitaka *et al.*, 2011; Kinoshita *et al.*, 2012). Each set of relative values was subsequently converted to a point in tetrahedral colour space with *X*, *Y* and *Z* coordinates using the following equations (Endler & Mielke, 2005).

For avian UVS vision (replace u with v for avian VS vision):

$$X = \frac{1 - 2s - m - u}{2}\sqrt{\frac{3}{2}}$$

$$Y = \frac{3m + u - 1}{2\sqrt{2}}$$

$$Z = u - \frac{1}{4}$$

For butterfly colour vision:

$$X = \frac{1 - 2b - g - u}{2}\sqrt{\frac{3}{2}}$$

$$Y = \frac{3g + u - 1}{2\sqrt{2}}$$

$$Z = u - \frac{1}{4}$$

Lastly, volume overlap was calculated to describe the extent to which two colour distributions overlap in colour space. The greater the overlap between two colour distributions, the more similar they are from the receiver's visual perspective. Volume overlap has been used as a measure of colour mimicry between cuckoo and host eggs, with the two showing increasingly overlap in avian colour space as hosts exhibit stronger rejection responses (Stoddard & Stevens, 2011).

Modelling of colour space and calculation of volume overlap were done using the R package *pavo* (Maia *et al.*, 2013).

Brightness perception

As mentioned above, colour space modelling does not consider the achromatic aspect of visual signals. Increasing evidence suggests that perception of colour and brightness are processed by different sets of photoreceptors (Osorio *et al.*, 1999a; Osorio & Vorobyev, 2005; Koshitaka *et al.*, 2011; Kinoshita *et al.*, 2012). Brightness perception in birds is believed to be mediated by the double cones (DC), which have a broad spectral sensitivity (Osorio *et al.*, 1999a, 1999b). In *Papilio xuthus*, brightness perception appears to involve the broadband (BB) receptors, which similarly have a wide spectral sensitivity (Koshitaka *et al.*, 2011).

To model perception of brightness from an avian or butterfly visual perspective, quantum catch was calculated as shown above. For avian perception, I used the spectral sensitivity of the double cone of the blue tit (*Cyanistes caeruleus*), which is an insectivorous bird with a well-studied visual system (Hart *et al.*, 2000b). Spectral sensitivity of the BB receptor of *Papilio xuthus* was provided by K. Arikawa. The illuminant 'D65', which represents standard daylight, was used as the irradiance spectrum.

According to Fechner's law, the intensity of the perceived stimulus is proportional to the logarithm of the actual stimulus intensity. Therefore, brightness perception (i.e., luminance) is given by the following equation (where i = DC or BB):

$$L = \ln q_i$$

Modelling was done using the R package *pavo* (Maia *et al.*, 2013).

Statistical Analyses

Shapiro-Wilk tests were used to check for normality of data. Luminance of males and females of sexually monomorphic *Papilio* species were compared using Wilcoxon signed-rank tests. All statistical analyses were conducted in R (R Core Team, 2013). Values are presented as mean±SD.

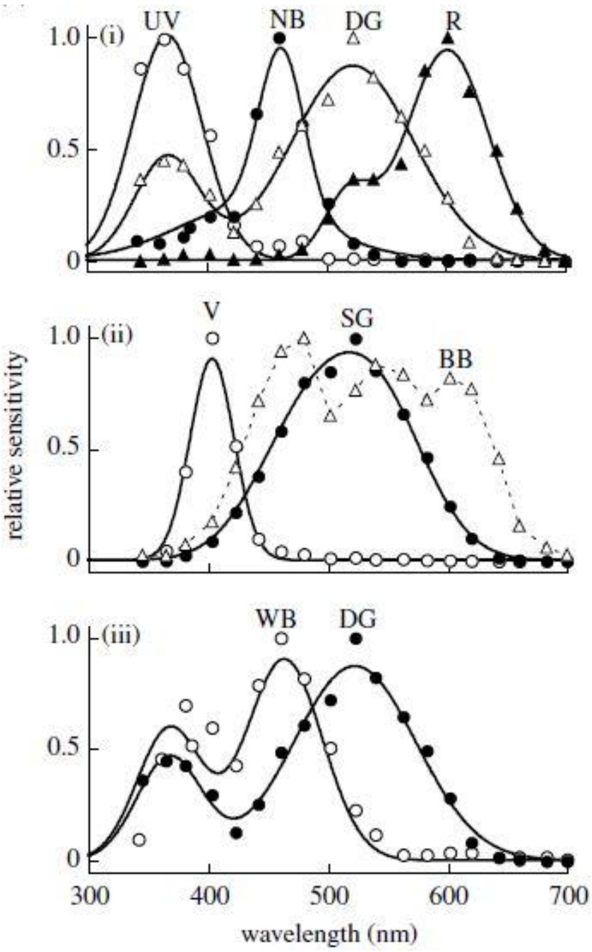

Figure 3.3. Spectral sensitivities of *Papilio xuthus* photoreceptors contained in each type of ommatidium: (i) type I; (ii) type II; (iii) type III. UV, ultraviolet; V, violet, NB, narrow-blue; WB, wide-blue; DG, double-peaked green; SG, single-peaked green; R, red; BB, broadband. Taken from Koshitaka et al. (2008).

RESULTS

Association between Sexual Dichromatism and Batesian Mimicry

A total of 60 *Papilio* butterfly species belonging to the subgenus *Menelaides* were scored for sexual dichromatism and presence and type of Batesian mimicry (Table 3.1). Twenty-four species were classified as sexually dichromatic, and among these species, all but one (i.e., *P. weymeri*) showed female-limited mimicry (but see comments below Table 3.1). Thirty-six species were considered to be sexually monochromatic based on wing colour patterns, out of which six species were mimetic (i.e., *P. bootes, P. dravidarum, P. janaka, P. lampsacus, P. macilentus, and P. protenor*) and the remaining 30 species were non-mimetic.

Reflectance and Visual Modeling of Butterfly Wing Colours

Wing reflectance measurements were obtained for 46 *Papilio* butterfly species (as indicated in Table 3.1), out of which 42 species had white patches on the dorsal and/or ventral hindwings of one or both sexes. Dorsal hindwing white patches of non-mimetic butterflies (i.e., both sexes of sexually monomorphic non-mimetic species and non-mimetic males of female-limited mimetic species) showed extremely low reflectance in the UV (300 to 400 nm), followed by high reflectance at longer wavelengths (~450 to 700 nm) (Figure 3.4). The only exception was the male of *P. jordani* (a female-limited mimetic species), which showed relatively high UV reflectance. Unlike dorsal white patches, ventral hindwing white patches of non-mimetic butterflies generally exhibited higher UV reflectance (Figure 3.4).

In contrast, dorsal and ventral hindwing white patches of mimetic butterflies (i.e., both sexes of sexually monomorphic mimetic species and mimetic females of female-limited mimetic species) showed a gradual increase in reflectance from 300 to 700 nm (Figure 3.5). Exceptions were the female of *P. castor* (a female-limited mimetic species) and both sexes of *P. dravidarum* (a sexually monomorphic mimetic species), whose white patches had very low UV reflectance (red lines in Figure 3.5).

Wing colouration was measured for a total of five toxic troidine butterfly species: *Pachliopta aristolochiae*, *P. pandiyana*, *Byasa dasarada*, *B. polyeuctes*, and *B. latreillei*. Their dorsal and ventral hindwing white patches exhibited a steady increase in reflectance from 300 to 700 nm (Figure 3.6).

Reflectance of hindwing white patches of non-mimetic, mimetic, and toxic butterflies were subsequently mapped in avian colour space to determine the extent of overlap in their colour distributions. The male of *Papilio jordani* was excluded because its reflectance differed from those of other non-mimetic butterflies, suggesting that it is potentially mimetic. The female of *P. castor* and both sexes of *P. dravidarum* were also excluded because they mimic *Euploea* spp., which have a different wing reflectance from troidine models.

The distribution of hindwing white patches of mimetic butterflies in avian colour space overlapped with that of models (Figure 3.7 and 3.8). The extent of volume overlap between mimics and models (expressed relative to volume occupied by models) was 90% and 80% for dorsal and ventral patches, respectively, from an avian UVS perspective, and 88% for both dorsal and ventral patches from an avian VS perspective. On the other hand, the distribution of hindwing white patches of non-mimetic butterflies in avian colour space did not overlap with that of toxic species (Figure 3.7 and 3.8).

In addition, there was no overlap between the distributions of dorsal white patches of mimetic and non-mimetic butterflies in avian colour space (Figure 3.7 and 3.8), whereas a small overlap was found for ventral patches (9% and 7% for avian UVS and VS vision, respectively; expressed relative to volume occupied by mimics).

Table 3.1. Sexual dichromatism and Batesian mimicry of *Papilio* (subgenus *Menelaides*) butterflies

Species	Sexual dichromatism	Batesian mimicry	Mimicry type
Papilio acheron	monochromatic	absent†	NA
Papilio aegeus	dichromatic	present	female-limited
Papilio agenor	dichromatic	present	female-limited#
Papilio albinus	monochromatic	absent	NA
Papilio alcmenor	dichromatic	present	female-limited#
Papilio alphenor	dichromatic	present	female-limited
Papilio ambrax	dichromatic	present	female-limited
Papilio amynthor	monochromatic	absent	NA
*Papilio artaphernes**	monochromatic	absent	NA
Papilio ascalaphus	dichromatic	present	female-limited
*Papilio biseriatus**	monochromatic	absent	NA
Papilio bootes	monochromatic	present	monomorphic
Papilio bridgei	dichromatic	present	female-limited
*Papilio canopus**	monochromatic	absent	NA
Papilio capaneus	monochromatic	absent	NA
Papilio castor	dichromatic	present	female-limited
Papilio chaon	monochromatic	absent	NA
*Papilio cilix**	monochromatic	absent	NA
*Papilio daksha**	monochromatic	absent	NA
Papilio deiphobus	dichromatic	present	female-limited
Papilio deiphontes	dichromatic	present	female-limited
Papilio diophantus	monochromatic	absent	NA
Papilio dravidarum	monochromatic	present	monomorphic
*Papilio enganius**	monochromatic	absent	NA
Papilio forbesi	dichromatic	present	female-limited
Papilio fuscus	monochromatic	absent	NA
Papilio gambrisius	dichromatic	present	female-limited
Papilio godeffroyi	monochromatic	absent	NA
Papilio helenus	monochromatic	absent	NA
*Papilio heringi**	monochromatic	absent	NA
Papilio hipponous	monochromatic	absent	NA
*Papilio hypsicles**	monochromatic	absent	NA
*Papilio hystaspes**	monochromatic	absent	NA
Papilio iswara	monochromatic	absent	NA
Papilio iswaroides	monochromatic	absent	NA
Papilio janaka	monochromatic	present	monomorphic
*Papilio javanus**	dichromatic	present	female-limited
Papilio jordani	dichromatic	present	female-limited

Table 3.1. (*continued*)

Species	**Sexual dichromatism**	**Batesian mimicry**	**Mimicry type**
Papilio lampsacus	monochromatic	present	monomorphic
Papilio lowii	dichromatic	present	female-limited
Papilio macilentus	monochromatic	present	monomorphic
*Papilio mangarinus**	monochromatic	absent	NA
Papilio mayo	dichromatic	present	female-limited
*Papilio memnon**	dichromatic	present	female-limited[#]
Papilio nephelus	monochromatic	absent	NA
*Papilio nicconicolens**	monochromatic	absent	NA
Papilio oenomaus	dichromatic	present	female-limited
Papilio phestus	dichromatic	present	female-limited
Papilio polymnestor	monochromatic	absent	NA
Papilio polytes	dichromatic	present	female-limited
Papilio prexaspes	monochromatic	absent	NA
Papilio protenor	monochromatic	present	monomorphic
Papilio ptolychus	monochromatic	absent	NA
*Papilio rumanzovia**	dichromatic	present	female-limited
Papilio sataspes	monochromatic	absent	NA
Papilio schmeltzi	monochromatic	absent	NA
Papilio thaiwanus	dichromatic	present	female-limited[#]
Papilio tydeus	dichromatic	present	female-limited
Papilio weymeri	dichromatic	absent	NA
Papilio woodfordi	monochromatic	absent	NA

NA, not applicable

* wing reflectance not measured

[†] species classified as non-mimetic in this study, but may actually be sexually monomorphic mimetic

[#] species classified as showing female-limited mimicry in this study, but may actually have sexually dimorphic mimicry

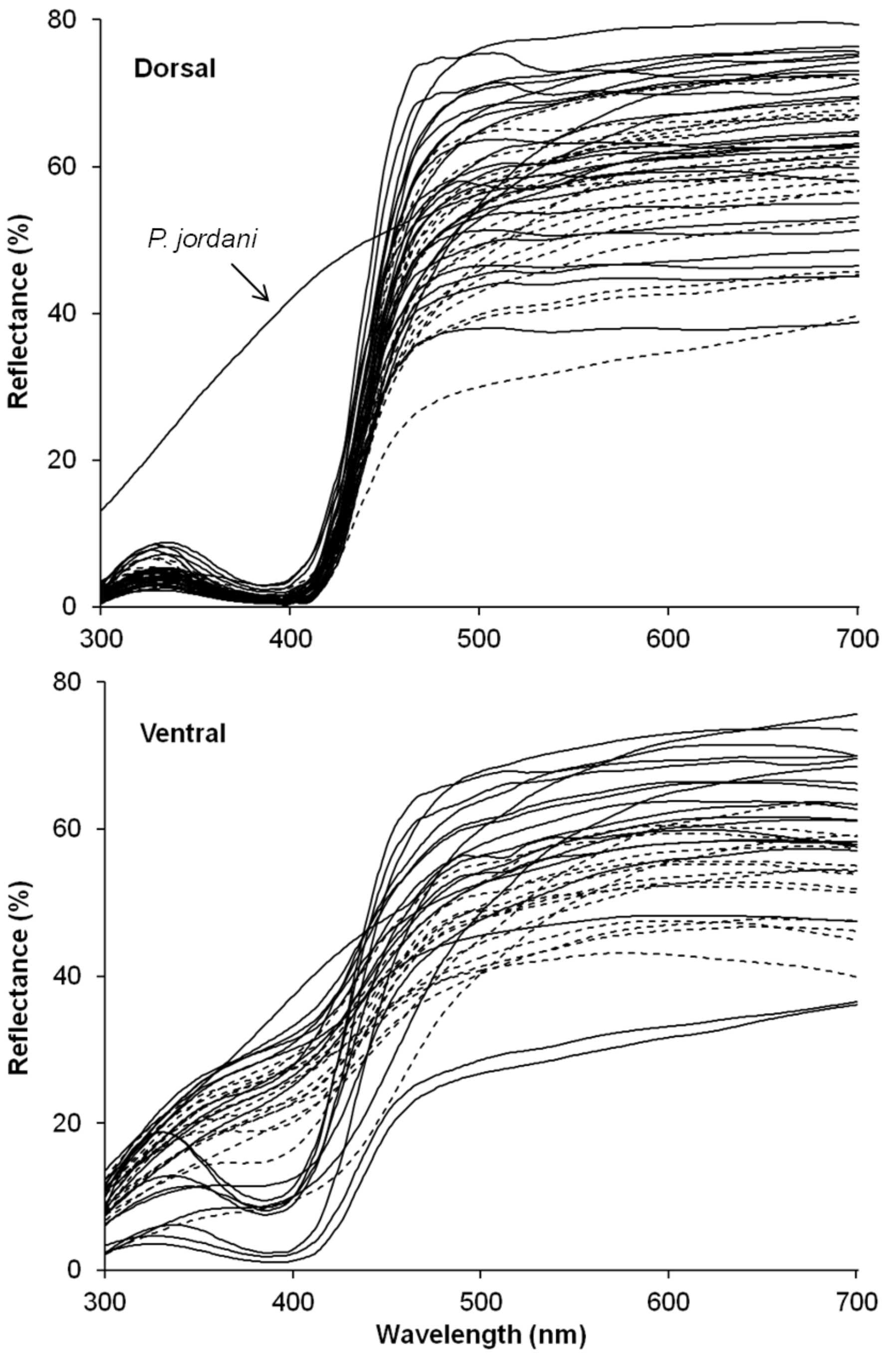

Figure 3.4. Reflectance of dorsal and ventral hindwing white patches of non-mimetic *Papilio* butterflies. Solid and dashed lines denote males and females, respectively.

Species (and sexes): *P. aegeus** (♂); *P. albinus* (♂♀); *P. alphenor* (♂♀); *P. ambrax** (♂); *P. amynthor* (♂♀); *P. bridgei** (♂); *P. capaneus* (♂♀); *P. castor* (♂); *P. chaon* (♂♀); *P. diophantus* (♂♀); *P. fuscus* (♂♀); *P. gambrisius** (♂); *P. godeffroyi** (♂♀); *P. helenus* (♂♀); *P. hipponous* (♂♀); *P. iswara* (♂♀); *P. iswaroides* (♂♀); *P. jordani* (♂); *P. nephelus* (♂♀); *P. phestus* (♂); *P. polytes* (♂♀); *P. prexaspes* (♂♀); *P. ptolychus** (♂♀); *P. sataspes* (♂♀); *P. schmeltzi** (♂♀); *P. tydeus** (♂); *P. weymeri* (♂); *P. woodfordi** (♂♀)

*dorsal only

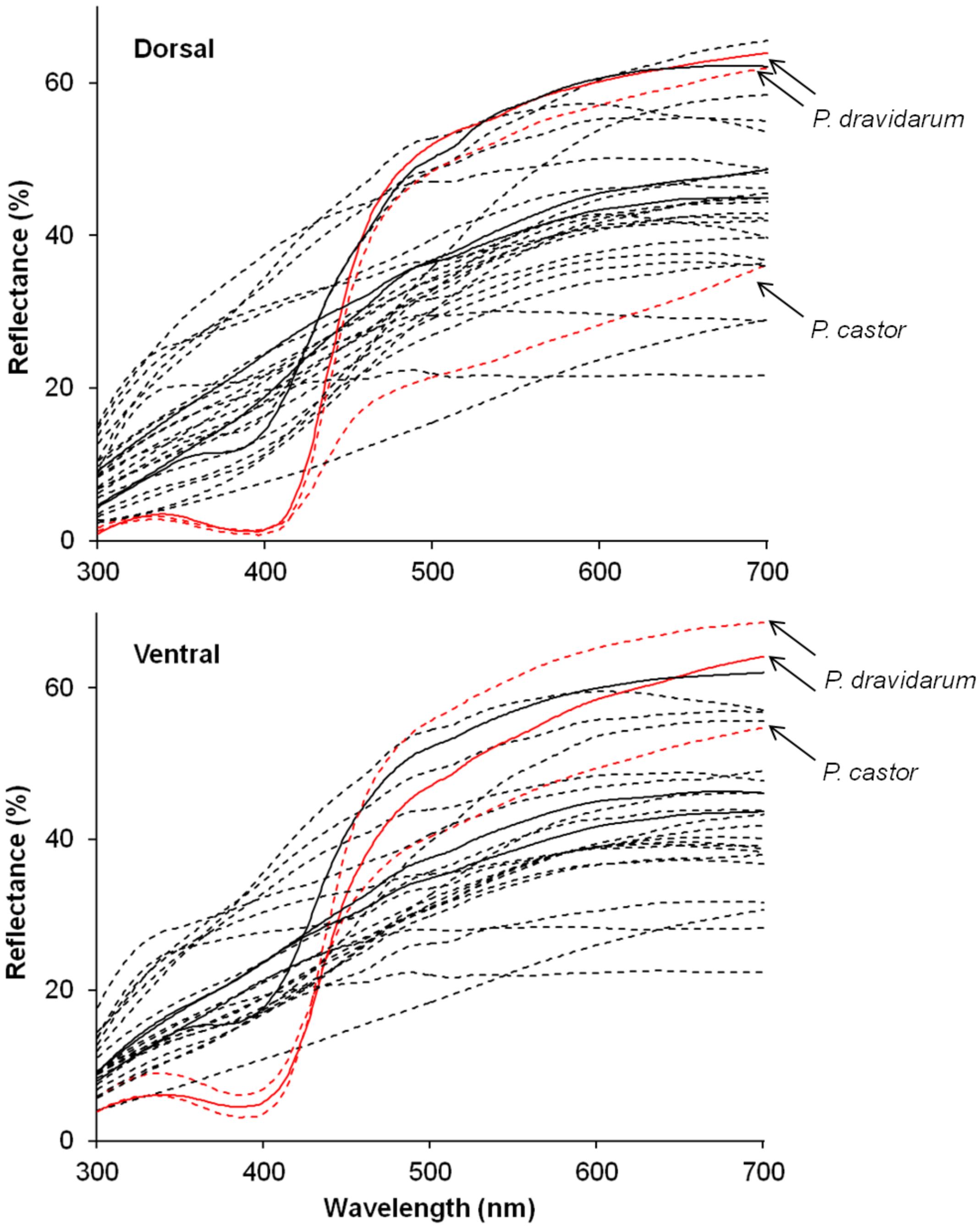

Figure 3.5. Reflectance of dorsal and ventral hindwing white patches of mimetic *Papilio* butterflies. Solid and dashed lines denote males and females, respectively. Red lines indicate exceptions (see text). Species (and sexes): *P. aegeus* (♀); *P. agenor* (♀); *P. alcmenor* (♀); *P. alphenor* (♀); *P. ambrax* (♀); *P. ascalaphus* (♀); *P. bootes* (♂♀); *P. castor* (♀); *P. deiphobus* (♀); *P. deiphontes* (♀); *P. dravidarum* (♂♀); *P. forbesi* (♀); *P. gambrisius* (♀); *P. janaka* (♂♀); *P. jordani* (♀); *P. lampsacus** (♂♀); *P. lowii* (♀); *P. mayo* (♀); *P. oenomaus* (♀); *P. phestus* (♀); *P. polytes* (♀); *P. thaiwanus* (♀); *P. tydeus* (♀)

*dorsal only

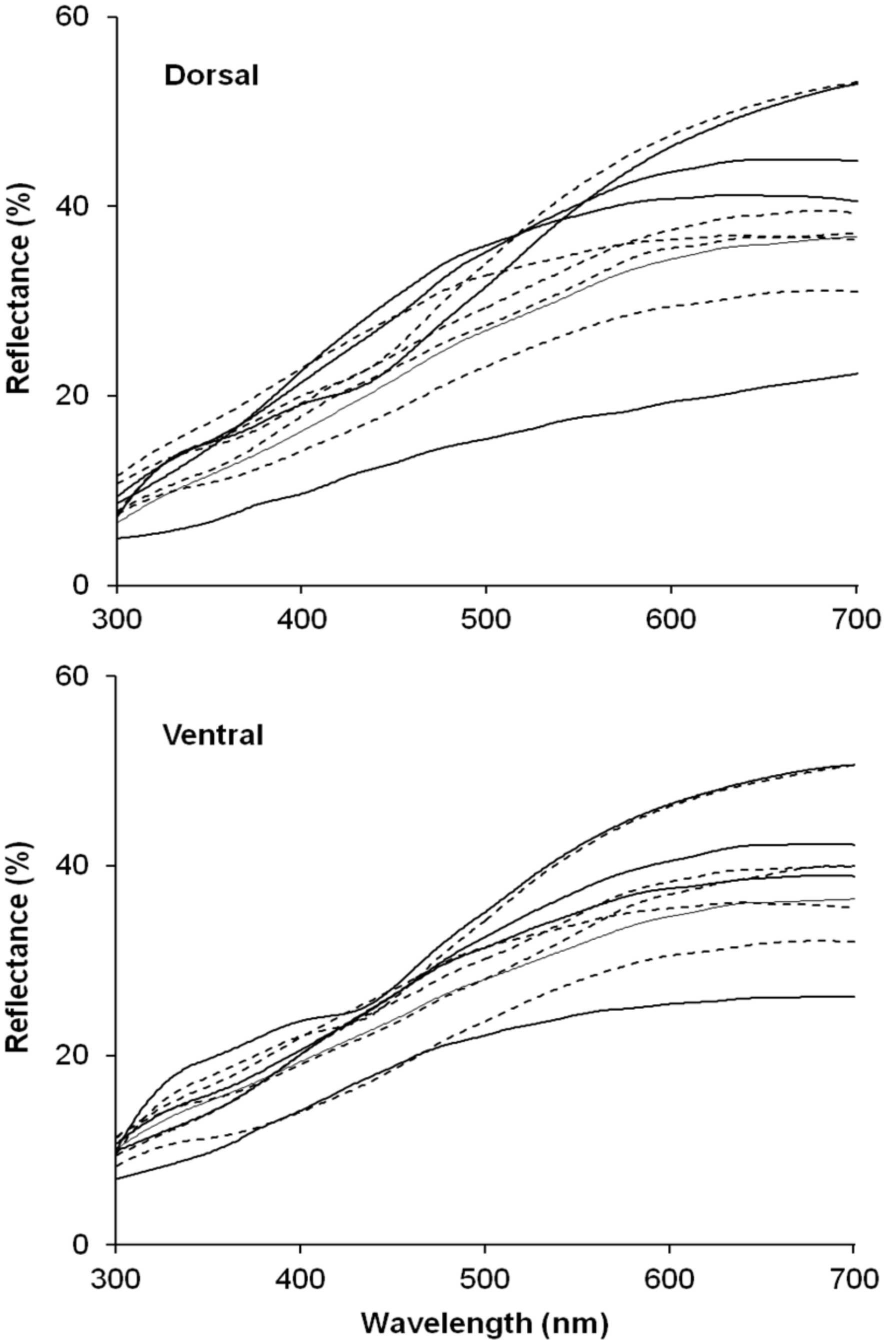

Figure 3.6. Reflectance of dorsal and ventral hindwing white patches of toxic butterflies which act as models for mimetic *Papilio* butterflies. Solid and dashed lines denote males and females, respectively. Species (and sexes): *Pachliopta aristolochiae* (♂♀); *P. pandiyana* (♂♀); *Byasa dasarada* (♂♀); *B. polyeuctes* (♂♀); *B. latreillei* (♂♀)

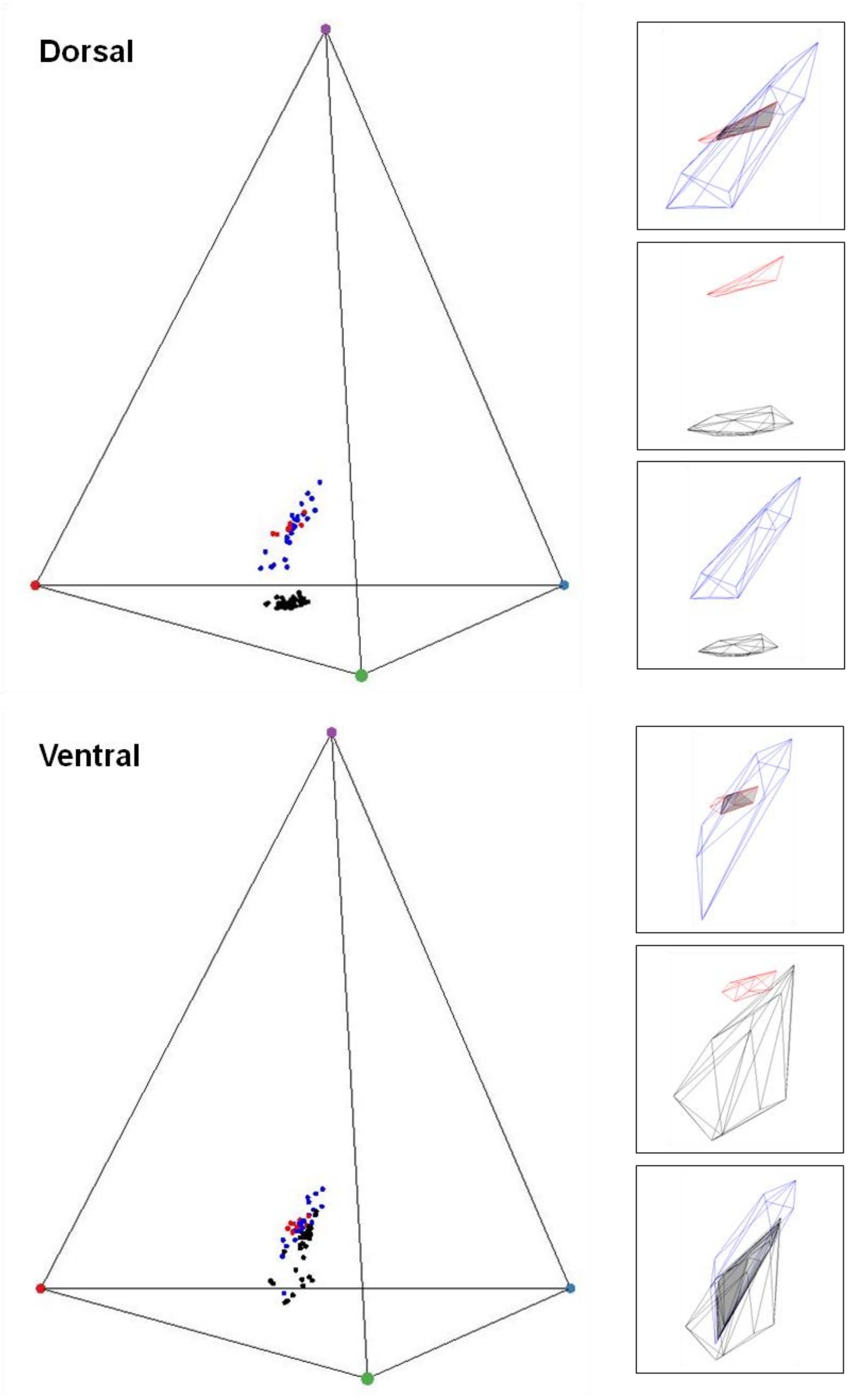

Figure 3.7. Distributions of dorsal and ventral hindwing white patches of mimetic (blue), non-mimetic (black), and toxic (red) butterflies in avian tetrahedral colour space (UVS). Vertices of tetrahedron represent the four avian cone types (purple: UVS; blue: SWS; green: MWS; red: LWS). Images on the right depict colour space occupied by mimetic, non-mimetic, and toxic butterflies, with colour space volume overlap (if any) shown in grey. Modeling was done using averaged avian UVS sensitivities.

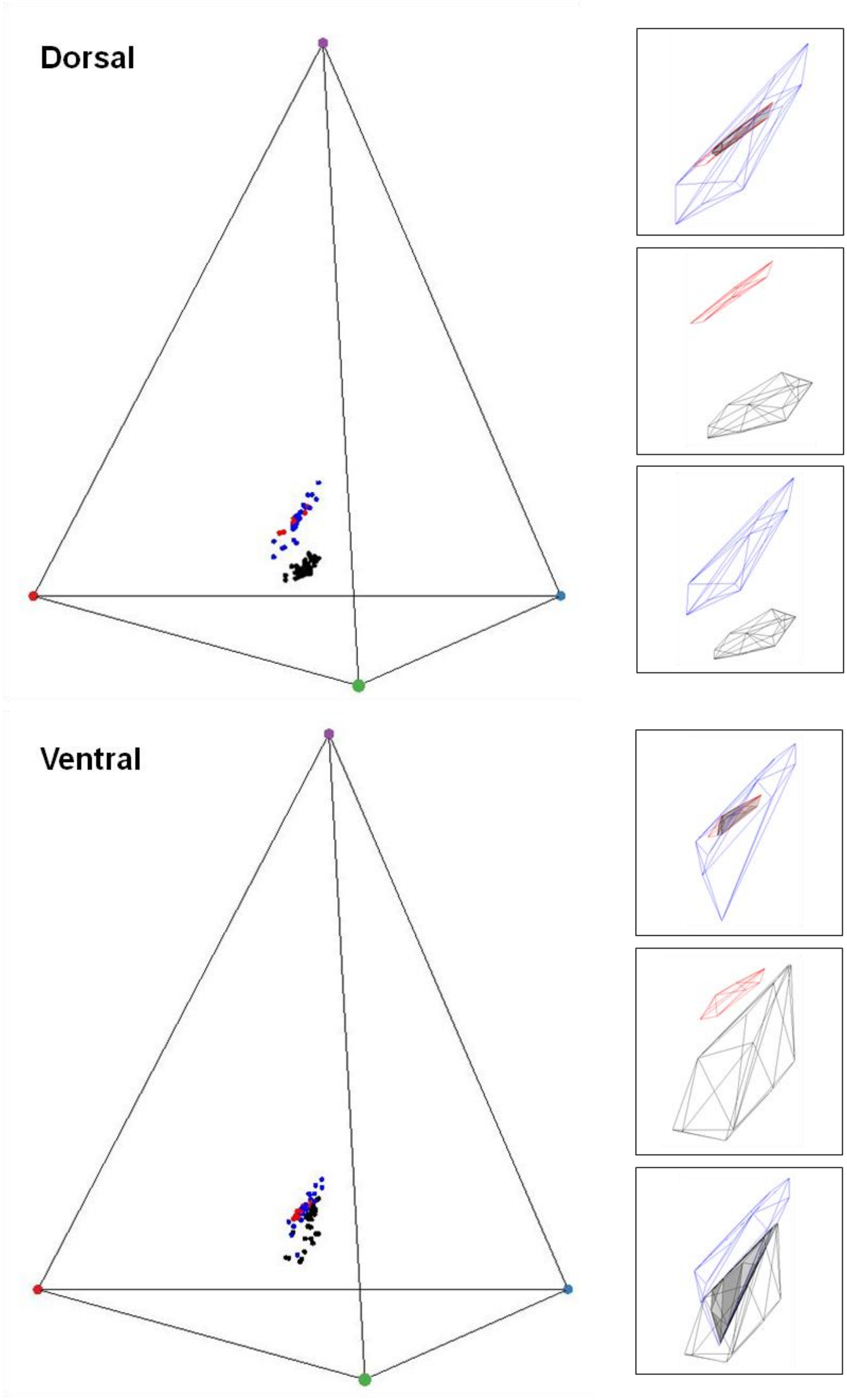

Figure 3.8. Distributions of dorsal and ventral hindwing white patches of mimetic (blue), non-mimetic (black), and toxic (red) butterflies in avian tetrahedral colour space (VS). Vertices of tetrahedron represent the four avian cone types (purple: VS; blue: SWS; green: MWS; red: LWS). Images on the right depict colour space occupied by mimetic, non-mimetic, and toxic butterflies, with colour space volume overlap (if any) shown in grey. Modeling was done using averaged avian VS sensitivities.

Sexual Dichromatism in Sexually Monomorphic Butterfly Species

Reflectance measurements revealed the presence of sexual differences in wing colouration of butterfly species with sexually monomorphic wing patterns (Figure 3.9). Visual modeling showed that the distributions of male and female wing colouration overlapped substantially in both butterfly and avian colour space (Figure 3.10), suggesting that male and female colours were similar in chromatic aspects. However, significant differences in luminance were found between the sexes from the visual perspective of butterfly conspecifics: males had brighter white hindwing patches (Wilcoxon signed rank test: dorsal [♂ = 3.95±0.19 vs. ♀ = 3.79±0.21], $V = 274$, $p < 0.001$, $N_{♂} = N_{♀} = 23$; ventral [♂ = 3.91±0.23 vs. ♀ = 3.79±0.15], $V = 152$, $p < 0.01$, $N_{♂} = N_{♀} = 18$) and darker black wing regions (Wilcoxon signed rank test: dorsal [♂ = −0.13±0.50 vs. ♀ = 0.44±0.55], $V = 7$, $p < 0.001$, $N_{♂} = N_{♀} = 25$; ventral [♂ = 0.05±0.52 vs. ♀ = 0.46±0.53], $V = 0$, $p < 0.001$, $N_{♂} = N_{♀} = 26$) than females (Figure 3.11). Similarly, the sexes showed significant differences in luminance from the visual perspective of avian predators, with males having brighter white hindwing patches (Wilcoxon signed rank test: dorsal [♂ = 4.06±0.19 vs. ♀ = 3.91±0.21], $V = 275$, $p < 0.001$, $N_{♂} = N_{♀} = 23$; ventral [♂ = 4.00±0.22 vs. ♀ = 3.88±0.16], $V = 152$, $p < 0.01$, $N_{♂} = N_{♀} = 18$) and darker black wing regions (Wilcoxon signed rank test: dorsal [♂ = −0.02±0.51 vs. ♀ = 0.58±0.57], $V = 6$, $p < 0.001$, $N_{♂} = N_{♀} = 25$; ventral [♂ = 0.17±0.54 vs. ♀ = 0.59±0.55], $V = 0$, $p < 0.001$, $N_{♂} = N_{♀} = 26$) than females (Figure 3.11).

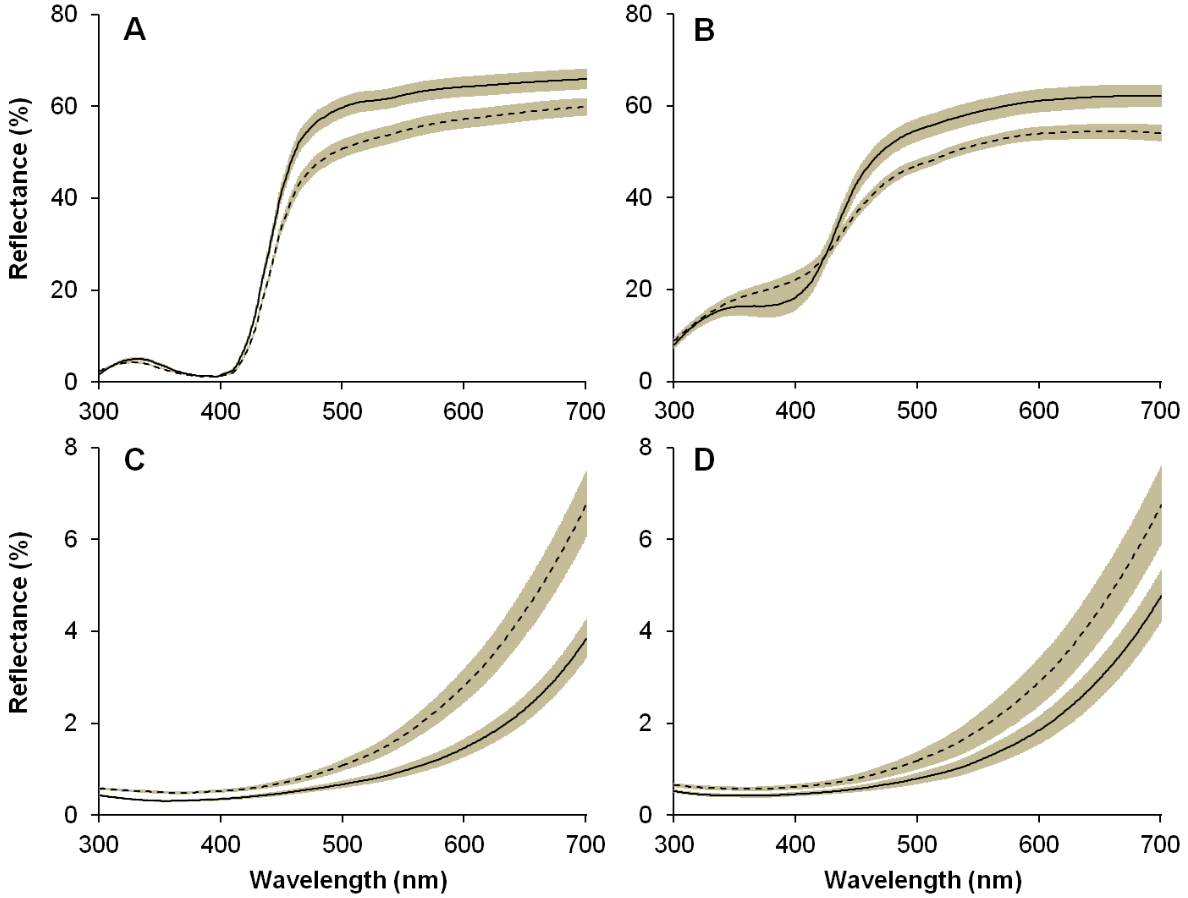

Figure 3.9. Reflectance spectra of wing colouration of males (solid lines) and females (dashed lines) of sexually monomorphic *Papilio* butterfly species. (A) dorsal hindwing white; (B) ventral hindwing white; (C) dorsal hindwing black; (D) ventral hindwing black. Lines and gray regions indicate mean and standard error, respectively.

Species: *P. acheron*[1]; *P. albinus*; *P. alphenor*; *P. amynthor*; *P. bootes*[2]; *P. capaneus*; *P. chaon*; *P. diophantus*; *P. dravidarum*; *P. fuscus*; *P. godeffroyi*[3]; *P. helenus*; *P. hipponous*; *P. iswara*; *P. iswaroides*; *P. janaka*[2]; *P. lampsacus*[2]; *P. macilentus*[2]; *P. nephelus*; *P. polymnestor*[2]; *P. polytes*; *P. prexaspes*; *P. protenor*[2]; *P. ptolychus*[3]; *P. sataspes*; *P. schmeltzi*[3]; *P. woodfordi*[4]

[1](D) only; [2](C) and (D); [3](A), (C) and (D); [4](A) only

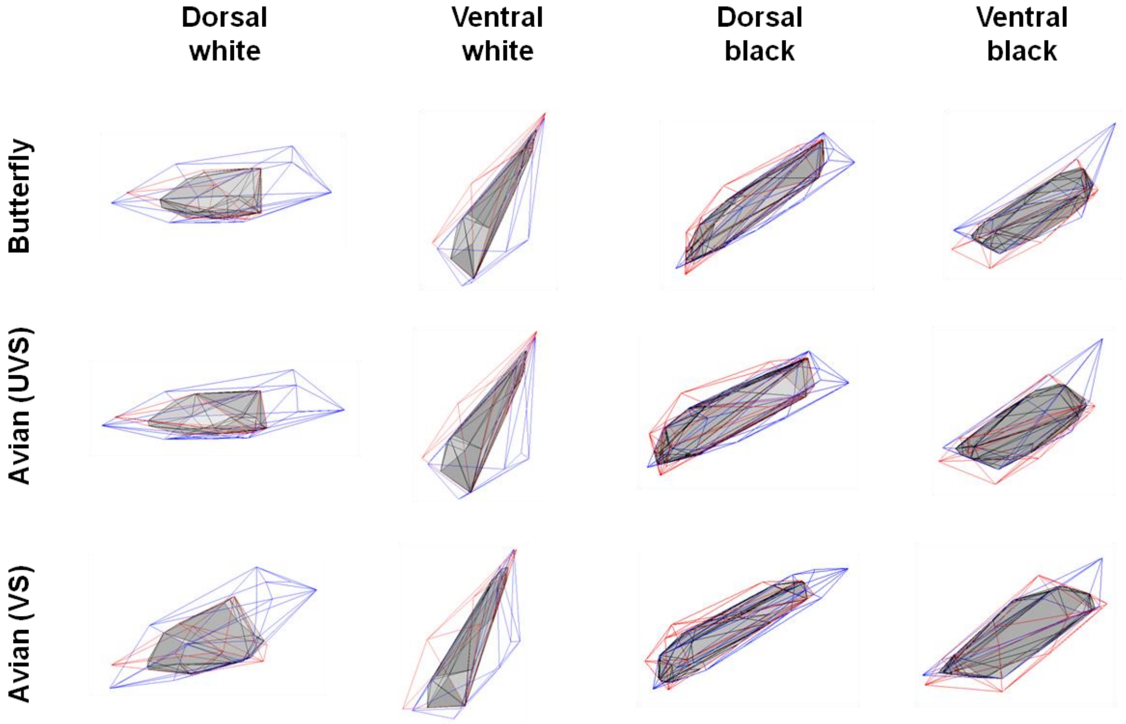

Figure 3.10. Distributions of male (blue) and female (red) wing colouration of sexually monomorphic *Papilio* butterfly species in butterfly and avian colour space, showing substantial overlap (grey regions) in all cases. Butterfly visual modeling was done using spectral sensitivities of *Papilio xuthus*, while avian visual modeling was performed using mean UVS and VS sensitivities. Refer to Figure 3.9 for list of species.

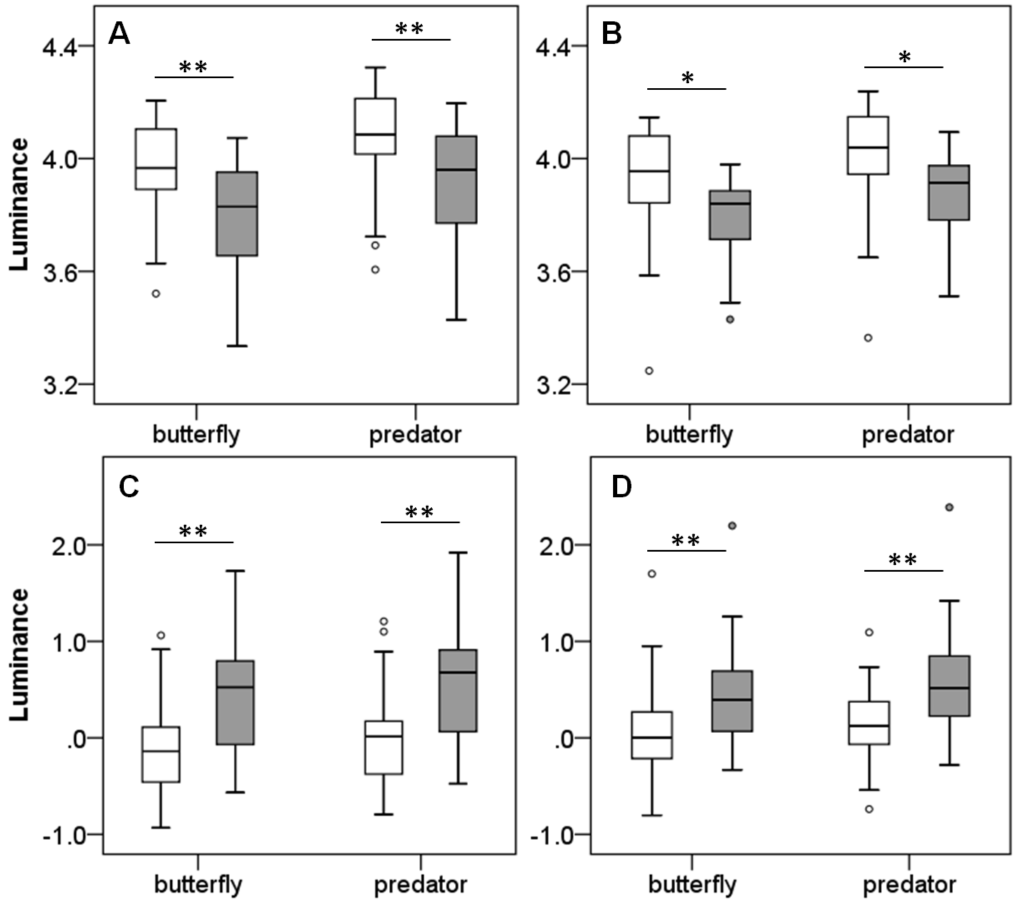

Figure 3.11. Boxplots showing differences in luminance between male (white) and female (grey) wing colouration of sexually monomorphic *Papilio* butterfly species from visual perspective of butterfly conspecifics and avian predators. (A) dorsal hindwing white; (B) ventral hindwing white; (C) dorsal hindwing black; (D) ventral hindwing black. Butterfly visual modeling was done using the broadband receptor spectral sensitivity of *Papilio xuthus*, and avian visual modeling was done using double cone sensitivity of the blue tit *Cyanistes caeruleus*. ** $p < 0.001$; * $p < 0.01$

Species: *P. acheron*[1]; *P. albinus*; *P. alphenor*; *P. amynthor*; *P. bootes*; *P. capaneus*; *P. chaon*; *P. diophantus*; *P. dravidarum*; *P. fuscus*; *P. godeffroyi*[2]; *P. helenus*; *P. hipponous*; *P. iswara*; *P. iswaroides*; *P. janaka*; *P. lampsacus*[2]; *P. macilentus*[3]; *P. nephelus*; *P. polymnestor*[3]; *P. polytes*; *P. prexaspes*; *P. protenor*[3]; *P. ptolychus*[2]; *P. sataspes*; *P. schmeltzi*[2]; *P. woodfordi*[4]

[1] (D) only; [2] (A), (C) and (D); [3] (C) and (D); [4] (A) only

As female butterflies are generally less frequently sighted than males in nature, specimens of female butterflies may be rarer and in poorer condition as compared to male specimens, which might be more abundant. Specimen condition could potentially affect wing reflectance due to wing scale loss (e.g., Kemp, 2006). To determine whether brightness differences found between males and females were caused by sexual differences in specimen condition, I also compared the wing reflectance of 20 freshly eclosed males and non-mimetic females of *Papilio polytes*. These butterflies were laboratory-reared and killed by freezing a few hours after eclosion when their wings had fully spread. Wing reflectance was measured (Figure 3.12) and visual modeling of luminance was performed as described in Methods.

Similar to the results obtained using museum specimens, significant differences in luminance were found between the sexes of *Papilio polytes* from the visual perspective of butterfly conspecifics: males had brighter white hindwing patches (independent t test: dorsal [♂ = 4.21±0.04 vs. ♀ = 4.03±0.04], $t_{38} = 12.76$, $p < 0.001$; ventral [♂ = 4.10±0.06 vs. ♀ = 3.92±0.03], $t_{38} = 11.42$, $p < 0.001$) and darker black wing regions (independent t test: dorsal [♂ = −0.84±0.17 vs. ♀ = −0.29±0.25], $t_{38} = -8.08$, $p < 0.001$; ventral [♂ = −0.18±0.21 vs. ♀ = −0.05±0.19], $t_{38} = -2.12$, $p < 0.05$) than females (Figure 3.13). The sexes also showed significant differences in luminance from the visual perspective of avian predators, with males having brighter white hindwing patches (independent t test: dorsal [♂ = 4.29±0.05 vs. ♀ = 4.14±0.04], $t_{38} = 10.87$, $p < 0.001$; ventral [♂ = 4.17±0.06 vs. ♀ = 4.01±0.03], $t_{38} = 9.93$, $p < 0.001$) and darker black wing regions (independent t test: dorsal [♂ = −0.76±0.18 vs. ♀ = −0.20±0.26], $t_{38} = -7.96$, $p < 0.001$; ventral [♂ = −0.10±0.23 vs. ♀ = 0.04±0.18], $t_{38} = -2.10$, $p < 0.05$) than females (Figure 3.13).

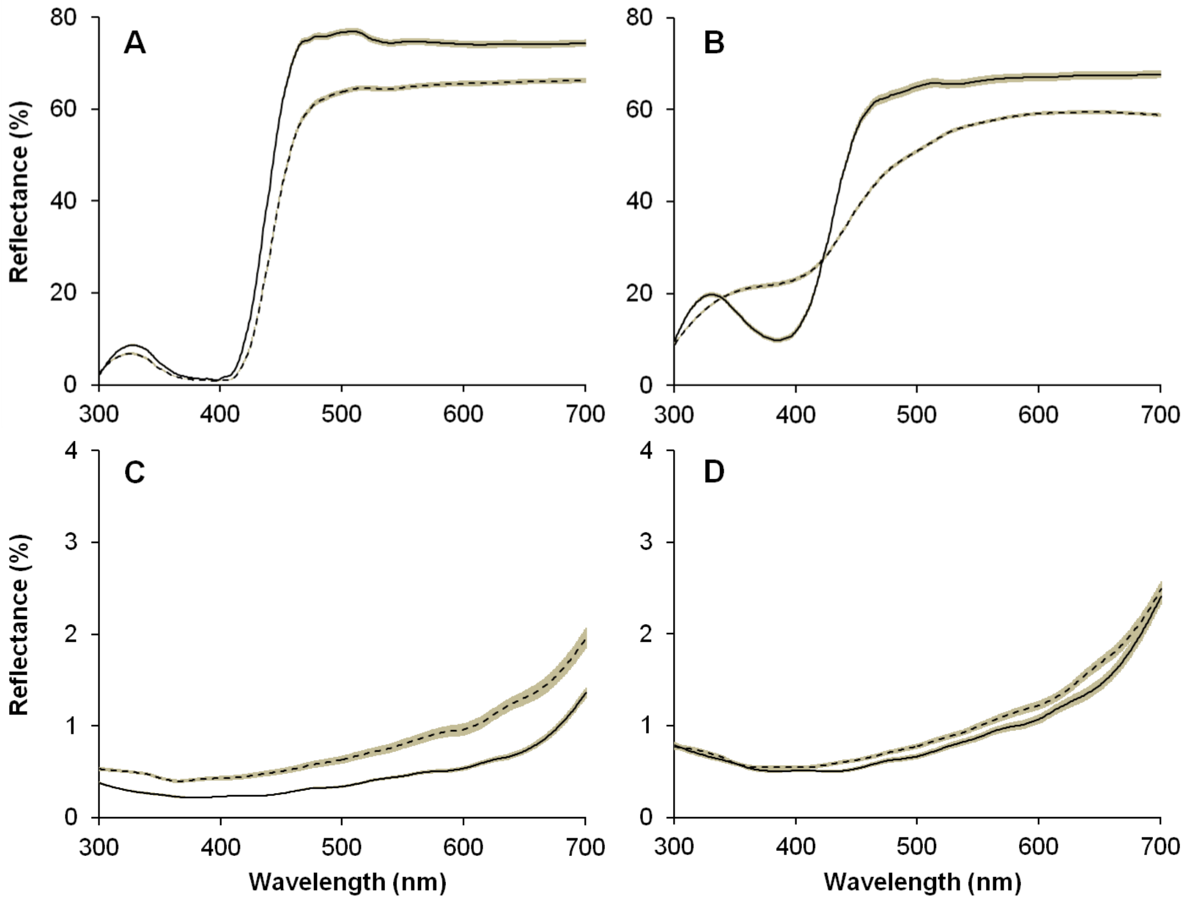

Figure 3.12. Reflectance spectra of wing colouration of males (solid lines) and non-mimetic females (dashed lines) of *Papilio polytes*. (A) dorsal hindwing white; (B) ventral hindwing white; (C) dorsal hindwing black; (D) ventral hindwing black. Lines and gray regions indicate mean and standard error, respectively ($N_{♂} = N_{♀} = 20$).

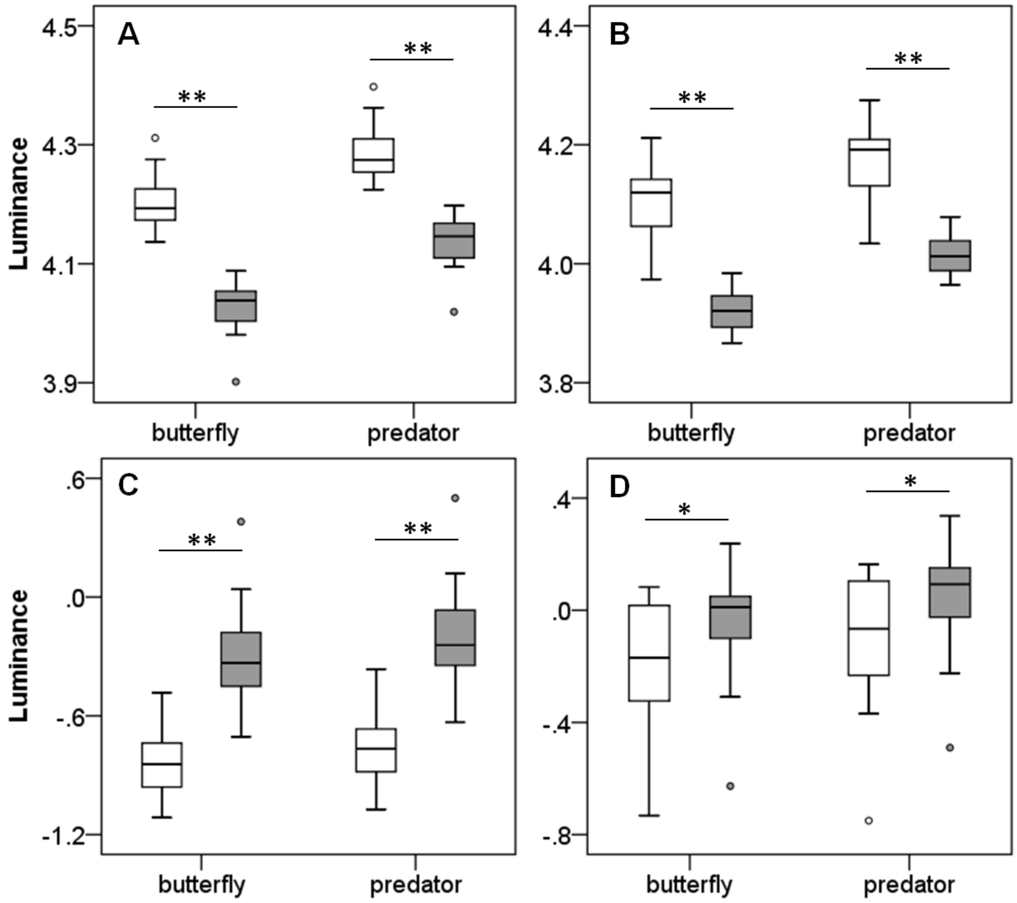

Figure 3.13. Boxplots showing differences in luminance between male (white) and non-mimetic female (grey) wing colouration of *Papilio polytes* from visual perspective of butterfly conspecifics and avian predators. (A) dorsal hindwing white; (B) ventral hindwing white; (C) dorsal hindwing black; (D) ventral hindwing black. Butterfly visual modeling was done using the broadband receptor spectral sensitivity of *Papilio xuthus*, and avian visual modeling was done using double cone sensitivity of the blue tit *Cyanistes caeruleus*. ** $p < 0.001$; * $p < 0.05$

DISCUSSION

Sexual dimorphism evolves due to selection pressures acting differentially on the two sexes, and can result from both natural and sexual selection (Stuart–Fox & Ord, 2004; Chenoweth *et al.*, 2008; Bell & Zamudio, 2012; Rojas & Endler, 2013). When colour traits show sexual dimorphism, males are usually the more conspicuous sex, due to stronger sexual selection on males and/or stronger natural selection on females (Andersson, 1994). However, when sexual dichromatism is the result of female-limited mimicry, females may be equally or more conspicuous than males because they mimic the aposematic wing colour patterns of their models. In this study, I investigated sexual dichromatism and Batesian mimicry in Mormon swallowtail butterflies (subgenus *Menelaides*). I found that sexual dichromatism in this group of *Papilio* butterflies appeared to be the evolutionary outcome of natural selection. Furthermore, avian visual modelling revealed that mimetic butterflies showed adaptive colour changes which are invisible to the human observer. In addition, butterfly species with sexually monomorphic wing patterns exhibited sexual differences in luminance which are potentially detectable by both butterfly conspecifics and avian predators.

Sexual Dichromatism and Batesian Mimicry

Sexual dichromatism in *Papilio* butterflies was found to be closely associated with female-limited mimicry in this study, suggesting that natural selection for Batesian mimicry in females drove the evolution of sexual dimorphism in this group of butterflies. This is in agreement with findings from a previous study, which showed that sexual dimorphism across several subgenera of *Papilio* butterflies was significantly correlated with female-limited Batesian mimicry (Kunte, 2008). Deviation of female phenotype towards protective colouration as a result of natural selection is also involved in the evolution of sexual dimorphism in other animals (Irwin, 1994; Burns, 1998; Penz & DeVries, 2002; Ord & Stuart-Fox, 2006). In addition, female-limited Batesian mimicry was more commonly observed than sexually monomorphic mimicry. This suggests that female butterflies may be under stronger selection for mimicry than male butterflies, possibly because females face higher predation risk than males (Ohsaki, 1995; Ide, 2006). It may also be due to stabilizing sexual selection on male wing colouration, resulting in males retaining non-mimetic wing patterns in female-limited mimetic species (Krebs & West, 1988; Lederhouse & Scriber, 1996). However, it is important to note that experimental evidence of Batesian mimicry in these butterfly species are scarce (but see Uesugi 1996), and behavioural studies involving avian predators are needed to verify the mimetic status of some of these butterflies.

Wing Colouration of Mimetic vs. Non-mimetic Butterflies

Many mimetic as well as non-mimetic *Papilio* butterflies have white patches on their dorsal hindwings which appear very similar in colour to the human observer. However, reflectance spectrometry revealed that the white patches of mimetic butterflies were UV-reflective (exceptions were the female of *P. castor* and both sexes of *P. dravidarum*, which mimic *Euploea* models), whereas those of non-mimetic butterflies reflected little or no UV (except for the male of *P. jordani*, which will be discussed below). Despite their drastically different spectral reflectance, wing patches of mimetic and non-mimetic butterflies appear to be of similar colour to human observers due to our inability to perceive UV. Since birds are sensitive to UV (Bennett & Cuthill, 1994), white patches of mimetic butterflies are 'bird white' as they stimulate all four avian cone classes in relatively similar proportions. On the other hand, those of non-mimetic butterflies are not 'bird white' and would instead appear as a strongly saturated hue to birds because SWS, MWS, and LWS cones are stimulated with little or no signal from the UVS/VS cone. Therefore, this finding highlights the importance of measuring colouration in an objective manner (i.e., using reflectance spectrometry) to avoid the biases of human vision.

While the hindwing white patches of mimetic *Papilio* butterflies had very different reflectance from those of non-mimetic butterflies, they were fairly similar to those of toxic butterflies (i.e., potential models). Mapping of the spectra of these three groups of butterflies in avian colour space showed that the wing reflectance of mimetic butterflies and toxic butterflies overlapped substantially, whereas those of non-mimetic butterflies did not overlap with the other two. The overlap in colour distributions between mimetic butterflies and potential models suggests that avian predators would be unable to discriminate between these two groups of colours. This implies that the change in wing colouration from UV-absorptive in non-mimetic butterflies to UV-reflective in mimetic butterflies (as shown by reflectance spectrometry) is an adaptive response to natural selection for Batesian mimicry. Further support was provided by two observations. First, in butterfly species showing female-limited mimicry, only the mimetic females become UV-reflective whereas non-mimetic males continue to show strong UV absorption, indicating that wing colour change was due to natural selection for mimicry (in sexually monomorphic mimetic species, both sexes become UV-reflective). Second, among mimetic butterflies, those which mimic *Pachliopta/Byasa* spp. have wing patches which are UV-reflective (i.e., 'bird white'), whereas those which mimic *Euploea* spp. (i.e., female of *Papilio castor* and both sexes of *P. dravidarum*) show low UV reflectance similar to that shown by non-mimetic butterflies. Unlike *Pachliopta* and *Byasa* butterflies, white patches on the hindwings of *Euploea* spp. (e.g., *E. core* and *E. sylvester*) reflect relatively little UV as compared to longer wavelengths (see Figure 2.3 in Chapter 2). Difference in wing reflectance between *Papilio* butterflies which mimic different model groups (i.e., *Pachliopta/Byasa* vs. *Euploea*) provides strong evidence that wing colour change in mimetic butterflies was the result of natural selection for Batesian mimicry of specific model species.

Reflectance spectra of hindwing white patches of non-mimetic *Papilio* butterflies were characteristic of papiliochrome II, which has been identified in several *Papilio* species (Umebachi, 1985). Although papiliochrome II is a fluorescent pigment, contribution of the fluorescence to the overall visual signal is relatively negligible (Wilts et al. 2012; but see Vigneron et al. 2008). Therefore, papiliochrome II functions primarily as a long-pass filter by absorbing UV and enhancing scattering of longer wavelengths. On the other hand, difference in reflectance and loss of fluorescence shown by white patches of mimetic *Papilio* butterflies suggest that papiliochrome II is absent in these butterflies. Nishikawa et al. (2013) found that the white wing regions in non-mimetic females of *Papilio polytes* contain kynurenine and N-β-alanyldopamine (NBAD), which are precursors of papiliochrome II, whereas these compounds are not detected in mimetic females. Furthermore, kynurenine and NBAD synthesis genes are upregulated in the white patches in non-mimetic females but not in mimetic females (Nishikawa *et al.*, 2013). These findings suggest that the adaptive colour change exhibited by mimetic butterflies (as shown in this study) is brought about by a change in the expression of genes involved in the synthesis of papiliochrome II. Thus, the evolution of Batesian mimicry in *Papilio* butterflies involves not just a change in the regions of gene expression, but more importantly, a change in gene activation.

All the non-mimetic *Papilio* butterflies in this study had dorsal hindwing white patches that reflected little or no UV. The only exception was the male of *P. jordani*, which had UV-reflective white patches that presumably do not contain papiliochrome II, suggesting that *P. jordani* males might actually be mimetic. However, they bear no phenotypic resemblance to any sympatric toxic butterfly species which could act as models. Another possibility concerns the huge difference in wing colour patterns between the mimetic females of *P. jordani* and the other mimetic *Papilio* butterflies. Unlike the other mimetic species which mimic *Pachliopta*/*Byasa*/*Euploea* spp., females of *P. jordani* are hypothesized to be Batesian mimics of *Idea* butterflies, which have wings that are mostly white in colour (cf. wings of *Pachliopta*/*Byasa*/*Euploea* butterflies which are mostly black or brown in colour). Therefore, in contrast to the other Batesian mimics, mimetic *P. jordani* females also have predominantly white wings that reflect UV. Furthermore, although papiliochrome II is not expressed in the white hindwing patches of mimetic butterflies, these butterflies typically also have some red or orange wing patches that are known to contain papiliochrome pigments (Umebachi, 1985; Koch *et al.*, 2000b; Wilts *et al.*, 2012a), implying that the papiliochrome synthesis pathway remains functional. However, mimetic *P. jordani* females do not possess these colour patches and hence may not express any genes involved in papiliochrome synthesis. As a result, expression of papiliochrome II in non-mimetic males may also be suppressed or inhibited due to some genetic correlations with the mimetic females.

Due to the absence of papiliochrome II, white hindwing patches of mimetic *Papilio* butterflies are ‘bird white’, unlike those of non-mimetic butterflies. As shown by visual modeling, this resulted in the segregation of non-mimetic butterflies from both mimetic butterflies and their toxic models in avian colour space. This could have important implications for the evolution of mimicry. Mimicry has been proposed to evolve via a two-step process, in which a large mutation first achieves approximate similarity to the model, after which smaller mutations improve the mimetic resemblance (Leimar *et al.*, 2012). Using theoretical simulations, Gamberale-Stille et al. (2012) showed that the evolution of

Batesian mimicry can be initiated when prey acquire a trait that is used by predators to categorize potential prey as unpalatable. If the trait is mainly controlled by a single locus, a single mutation could produce a large change in prey appearance as perceived by predators, shifting the mutant into the same category (i.e., unpalatable prey) as the model. Therefore, in the case of *Papilio* butterflies, if white wing patches form an important component of the aposematic signal of the models, loss of papiliochrome II expression could act as the first step in the evolution of Batesian mimicry in these butterflies. This postulation is supported by studies showing that avian predators exhibit prey generalization based on colour (Exnerová *et al.*, 2006; Ham *et al.*, 2006; Aronsson & Gamberale-Stille, 2008; Svádová *et al.*, 2009). Moreover, wing phenotypes of mimetic *Papilio* species reported to date are determined by a single gene locus (Koch *et al.*, 2000b; Kunte *et al.*, 2014; Timmermans *et al.*, 2014). Behavioural experiments involving avian predators and the manipulation of prey phenotype would be required to test this hypothesis.

White patches on the ventral hindwings of non-mimetic *Papilio* butterflies reflected relatively more UV than those on dorsal hindwings. As the ventral hindwing white patches of mimetic *Papilio* butterflies were also UV-reflective, this resulted in a small overlap in distributions of ventral white between mimetic and non-mimetic butterflies when mapped in avian colour space. The reduction in UV absorption suggests that lower quantities of papiliochrome II are present in ventral white patches as compared to dorsal patches of non-mimetic butterflies. Similarly, Umebachi (1977) found that white scales from the dorsal hindwings of male *P. helenus* and *P. castor* contained papiliochrome pigments, whereas those on the ventral hindwings did not. However, as most of these butterflies have very similar wing patterns on both sides of the wings, the reason as to why papiliochrome II is expressed in the dorsal wing patches but less so in ventral patches is unknown. Since the dorsal wing surface is exposed and visible during flight which is when intraspecific interactions occur, dorsal wing colour patterns may play a more important role in sexual signaling, such as conspecific recognition and mate choice, than ventral wing colour patterns. The presence of greater quantities of papiliochrome II in the dorsal white patches suggests that this pigment and its associated colour may be important components of a visual signal that is used in intraspecific signaling in *Papilio* butterflies.

Sexual Dichromatism in Sexually Monomorphic Butterfly Species

Among *Papilio* butterflies with sexually monomorphic wing patterns, reflectance spectrometry revealed that white hindwing patches of males had higher reflectance than those of females, while black hindwing regions of males had lower reflectance (i.e., stronger absorption) than those of females. Visual modeling showed that wing colouration of males and females overlapped substantially in both butterfly and avian colour space, suggesting that they appear very similar (in chromatic aspects) to conspecifics and predators. On the other hand, modelling of brightness perception from the visual perspectives of butterfly conspecifics and avian predators revealed sexual differences, with males having brighter white patches and darker black regions on their hindwings as compared to females. The same results were obtained using fresh specimens of *P. polytes*, indicating that the detected sexual differences in luminance were not an artifact caused by museum specimens of female butterflies being in poorer condition than those of males (a possible scenario since females are less frequently sighted and hence captured in nature). Therefore, *Papilio* butterfly species with sexually monomorphic wing colour patterns are potentially sexually dimorphic in terms of brightness to butterfly conspecifics and avian predators.

The sexual differences in luminance quantified in this study may be due to sexual selection acting on male wing colouration. Females of several butterfly species prefer to mate with males having brighter colours (Robertson & Monteiro, 2005; Kemp, 2007, 2008b; Papke *et al.*, 2007; Morehouse & Rutowski, 2010), and hence female mate choice could lead to the evolution of stronger achromatic contrast shown by male butterflies. Alternatively, duller white wing patches in females may be the evolutionary outcome of natural selection. Female butterflies may benefit from being less conspicuous to predators as they are vulnerable during oviposition and have a less effective escape flight due to their heavy egg-loads (Srygley & Chai, 1990; Srygley & Dudley, 1993). Another possible explanation is that the sexes may differ in resource allocation between reproduction and pigment production. The brighter white patches and darker black regions of male wings suggest that they contain greater quantities of papiliochrome II and melanin, respectively, than female wings. Both papiliochrome and melanin are nitrogen-rich pigments (Koch *et al.*, 2000a; Talloen *et al.*, 2004). However, nitrogen is also required for reproduction, especially egg production in females (Karl *et al.*, 2007; Geister *et al.*, 2008). Since nitrogen acquisition during the larval stage is limited (Slansky & Feeny, 1977), it is highly possible that a resource (i.e., nitrogen) trade-off exists between reproduction and pigment synthesis during wing development in the pupal stage. As male butterflies allocate less nitrogen to reproduction than females (Boggs, 1981; Karlsson & Wickman, 1990), they could invest comparatively more nitrogen in the formation of papiliochrome and melanin pigments, which would result in the observed sexual differences in luminance.

Future Studies

Evolution of different mimicry types

Currently, studies using molecular phylogenetic approaches to test the evolution of butterfly mimicry are scarce (Mullen, 2006; Kunte, 2008, 2009a; Prudic & Oliver, 2008). Therefore, a phylogenetic framework could be used to study the evolutionary relationships between (a) sexual monomorphism and dimorphism; (b) sexual dimorphism and female-limited Batesian mimicry; and (c) sexually monomorphic, female-limited, and sexually dimorphic Batesian mimicry in this subgenus (*Menelaides*) of swallowtail butterflies. Phylogenetic analyses of sexual dimorphism evolution using the butterfly genera *Bicyclus* and *Junonia* found that lineages displaying sexual dimorphism were more likely to become sexually monomorphic than they were to remain dimorphic (Oliver & Monteiro, 2011). Given the protective advantage of Batesian mimicry and its negative frequency-dependent nature, it would be informative to investigate whether an opposite trend is observed in *Papilio* butterflies, i.e., are lineages displaying sexual dimorphism (as a result of female-limited mimicry) more likely to remain so than to become sexually monomorphic (either mimetic or non-mimetic)? Other possible hypotheses to test using a phylogenetic approach include whether sexually monomorphic mimicry is evolutionarily ancestral to female-limited or sexually dimorphic mimicry, and also whether female-limited mimicry has evolved only in particular lineages. These findings will improve our understanding of the evolution and diversity of mimicry in swallowtail butterflies, as well as the selection pressures acting on butterfly wing colour patterns.

Wing scale structural mimicry

In this study, white hindwing patches of mimetic and non-mimetic *Papilio* butterflies were shown to have different reflectance due to the absence of papiliochrome II in mimetic butterflies. In addition to changes in pigment expression and wing reflectance, it may be worthwhile to examine whether the white wing scales of mimetic and non-mimetic butterflies exhibit any structural differences, and whether these differences, if present, are also an adaptive response to natural selection for mimicry. Wing scale structural mimicry has been found in the female-limited mimetic butterfly species *Hypolimnas anomala*, whose females are Batesian mimics of *Euploea mulciber* males (Saito, 2002). Both the cover and basal wing scales of non-mimetic males have a vastly different structure from those of the model. On the other hand, the cover scales of mimetic females have a structure that closely resembles that of the model, whereas the structure of basal scales is identical to that of non-mimetic males, suggesting strongly that structural changes shown by cover scales are the result of natural selection for Batesian mimicry in females. As several *Papilio* butterfly species in this study show female-limited Batesian mimicry, they are ideal subjects to investigate whether mimetic females exhibit wing scale structural mimicry, which is predicted to be absent in the non-mimetic males.

Implications for sexual selection

In *Papilio* species with female-limited Batesian mimicry, phylogenetic evidence indicates that female wing colour patterns have diverged from the ancestral phenotype (shown by non-mimetic males) to resemble those of toxic species (Kunte, 2008). As butterfly wing colouration play a role in intraspecific signaling (Kemp & Rutowski, 2011), the evolution of mimetic wing patterns in females may have implications for sexual selection. For female-polymorphic species with both mimetic and non-mimetic female forms (e.g., *P. polytes*), it has been hypothesized that, whereas mimetic female forms are favoured by natural selection, non-mimetic female forms are maintained by male mate choice (Burns, 1966). However, empirical evidence is largely lacking and existing studies used surrogate measures (e.g., spermatophore counts) which might not be an accurate representation of male preference (Burns, 1966; Pliske, 1972; Platt *et al.*, 1984; Cook *et al.*, 1994). To investigate whether male butterflies prefer to mate with non-mimetic or mimetic females, behavioural experiments offering males a choice of both mimetic and non-mimetic females should be conducted, and the occurrence of mating should be used as a direct measure of male mate preference. Furthermore, among *Papilio* species with sexually monomorphic wing patterns, males have brighter white hindwing patches than females, suggesting that these wing patches may play a role in sexual signaling. Brightness of these wing patches can be experimentally reduced to investigate whether female butterflies prefer to mate with males with brighter white patches, and thus whether the brighter white patches of male butterflies are the result of sexual selection via female mate choice.

CHAPTER 4

MATE CHOICE AND ITS EVOLUTIONARY IMPLICATIONS IN A *PAPILIO* BUTTERFLY

ABSTRACT

Sexual selection via mate choice has been implicated in the evolution of sexual dimorphism and female polymorphism. Although mate choice based on wing colouration has been documented in butterflies, empirical evidence remains scant and is limited to a small handful of species. In this study, I conducted behavioural experiments under greenhouse conditions to investigate female and male mate choice in the swallowtail butterfly *Papilio polytes*, which exhibits female-limited mimetic polymorphism. Males possess brighter white bands on their dorsal hindwings than do non-mimetic females, which could result from directional selection by females. However, mating assays showed that both mimetic and non-mimetic females did not discriminate between males with artificially-reduced brightness and those with intact brightness. On the other hand, male size seemed to influence female mate choice. As there was a positive correlation between male size and dorsal brightness, female preference for larger males might have led to the evolution of sexual dimorphism in brightness. In the second part of this study, I offered male butterflies a choice of mimetic and non-mimetic females, and found that males mated preferentially with the mimetic female form. Therefore, the hypothesis that male mate choice may be involved in the persistence of the non-mimetic female form was not supported. Future studies could investigate the role of female mate choice in the evolution of female-limited mimicry, as well as potential factors (e.g., female pheromones) that affect male mate choice.

INTRODUCTION

Sexual selection, first proposed by Darwin about 150 years ago (Darwin, 1871), generates non-random variation in reproductive success through two main mechanisms: intersexual mate choice and intrasexual competition for mates (Andersson, 1994). Mate choice occurs when individuals are more likely to mate with members of the opposite sex possessing certain traits, which could result in the evolution of exaggerated traits and sexual dimorphism (Andersson, 1994; Kokko *et al.*, 2003b). Selection for these mating preferences may be maintained due to direct fitness benefits that increase the survival or fecundity of the individual (Wagner, 2011), and/or due to indirect genetic benefits that increase offspring viability or attractiveness (Kotiaho & Puurtinen, 2007). In addition, mating preferences might be caused by sensory biases that result from natural selection on the physiology of sensory systems (Fuller *et al.*, 2005). Female mate choice is generally thought to be more prevalent than male mate choice due to the greater parental investment by females in most species (Andersson, 1994). However, males of some species invest substantially in mating effort (e.g., costly ejaculates or lengthy courtship) or parental care, which could lead to the evolution of male mate choice (Bonduriansky, 2001; Edward & Chapman, 2011).

With their highly diverse and often sexually dimorphic colour patterns, butterflies are ideal subjects to study the adaptive significance of colouration in sexual selection (Kemp & Rutowski, 2011). Female mate choice based on male wing colouration has been shown in several butterfly species (Robertson & Monteiro, 2005; Davis *et al.*, 2007; Kemp, 2007, 2008b; Papke *et al.*, 2007; Morehouse & Rutowski, 2010; Rutowski & Rajyaguru, 2012; Kemp *et al.*, 2014). However, some studies of female mate choice involved the entire removal or substitution of male wing colour patterns, followed by noting the mating success of males with normal versus altered phenotypes (e.g., Silberglied & Taylor 1978; Rutowski & Rajyaguru 2012). Thus, it is unclear if female response to the absence/presence of a male trait signifies actual mate choice, which requires assessment among conspecific males, or was simply the result of interference with mate recognition, which involves the discrimination of conspecifics from heterospecifics (Ryan & Rand, 1993; Pfennig, 1998; Phelps *et al.*, 2006). To resolve this problem, recent studies have employed graded manipulations of wing colouration or made use of natural variation shown by wild-collected males, and successfully demonstrated that female butterflies are able to discriminate among males based on intraspecific variation in colour signals (e.g., Robertson & Monteiro 2005; Kemp 2007a, 2007b; Morehouse & Rutowski 2010). Furthermore, female wing colouration also influences mate choice in male butterflies (Emmel, 1972; Knüttel & Fiedler, 2001; Ellers & Boggs, 2003; Kemp & Macedonia, 2007).

Papilio polytes is a common species of swallowtail butterfly widely distributed across India, Southeast Asia, Taiwan, as well as parts of China and Japan. Males are monomorphic and non-mimetic in all populations, whereas females exhibit polymorphic mimicry, with non-mimetic females having a male-like wing pattern (form *cyrus*), while mimetic females resemble toxic sympatric butterfly models belonging to the genus *Pachliopta* (Clarke & Sheppard, 1972; Uésugi, 1996). In India, three female forms of *Papilio polytes* can be found, consisting of the non-mimetic form *cyrus* and two mimetic female forms, *stichius* and *romulus*, which are Batesian mimics of *Pachliopta aristolochiae* and *Pachliopta hector*, respectively (Figure 4.1). The entire wing pattern is controlled by a single autosomal locus (Kunte *et al.*, 2014), and female polymorphism results from multiple alleles in a dominance hierarchy, whereby *romulus* is dominant over *stichius* and *cyrus*, while *cyrus* is recessive to *stichius* (Clarke & Sheppard, 1972).

As *Papilio polytes* exhibits female-limited mimicry, wing patterns of males and mimetic females are sexually dimorphic, with males displaying the ancestral wing colouration (Kunte, 2008). Furthermore, although males and females of form *cyrus* have similar wing patterns, reflectance spectrometry revealed that the white patches on the dorsal hindwings of males are brighter than those of females (Figure 4.2). Specifically, males reflect more visible wavelengths (~ 400 to 700 nm) than females, while reflectance in the ultraviolet (UV; 300 to 400 nm) is very low in both sexes due to the presence of UV-absorbing papiliochrome pigments in the wing scales (Umebachi, 1985). Since *Papilio* butterflies are able to perceive UV (Kinoshita & Arikawa, 2014), the absorption of UV and strong scattering of longer wavelengths should result in a highly conspicuous colour (which appears white to humans due to our insensitivity to UV) against the black ground colour of the hindwings. The presence of sexual dichromatism in this wing colour trait suggests that it may play a role in female mate choice.

Due to their resemblance to unpalatable toxic butterflies, mimetic female forms of butterfly species with female-limited mimetic polymorphism (e.g., *Papilio polytes*) suffer from lesser avian predation than non-mimetic females (Jeffords *et al.*, 1979; Ohsaki, 1995). As natural selection favours mimetic females, sexual selection via male mate choice has been proposed as a balancing force that helps to maintain the non-mimetic female form in the population (Burns, 1966). However, the hypothesis that males prefer to mate with non-mimetic females has been investigated in only two butterfly species, and with inconsistent findings: males of *Papilio glaucus* were not found to exert differential mate selection between mimetic and non-mimetic females (Pliske 1972; Platt et al. 1984; but see Burns 1966), whereas *Papilio dardanus* males were more likely to approach the mimetic female form (Cook *et al.*, 1994). Furthermore, these studies relied on surrogates such as spermatophore counts or number of male approaches, which may not be reliable indicators of male mate preference. Therefore, more empirical research is needed to elucidate male preference for different female forms in polymorphic butterflies.

In the present study, I investigated female and male mate choice in the butterfly *Papilio polytes* by conducting behavioural experiments using laboratory-reared butterflies. The brighter white bands on the dorsal hindwings of males could have evolved under directional selection by females. Thus, using a graded manipulation of male dorsal brightness, I evaluated whether females prefer to mate with males showing intact brightness than with those having reduced brightness. Mating preferences of both mimetic (form *romulus*) and non-mimetic (form *cyrus*) females were determined. In the second part of this study, I tested the hypothesis that male mate choice is involved in the maintenance of the non-mimetic female form. I examined whether males exhibit any mating preference for non-mimetic versus mimetic females by offering them a choice of both female forms (*cyrus* and *romulus*). The significance and implications of these findings were discussed, and promising avenues for future research were also proposed.

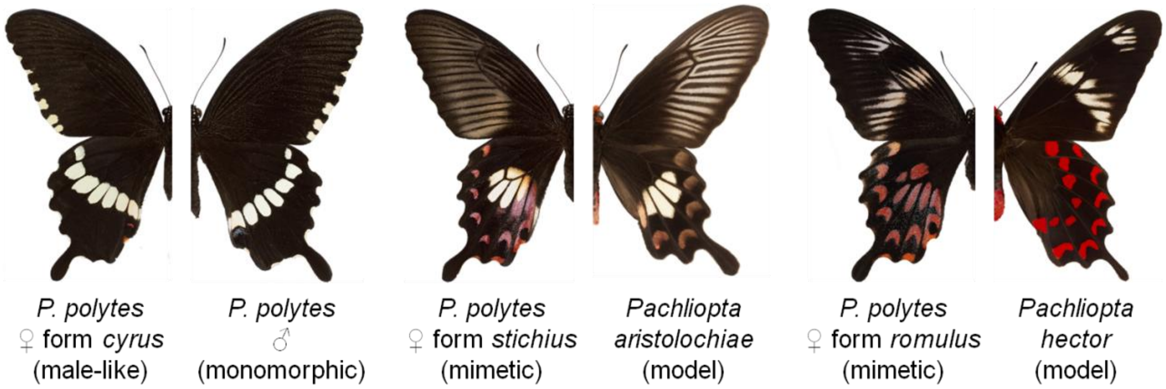

Figure 4.1. *Papilio polytes* exhibits female-limited mimetic polymorphism, with three female forms (one non-mimetic and two mimetic) occurring in India.

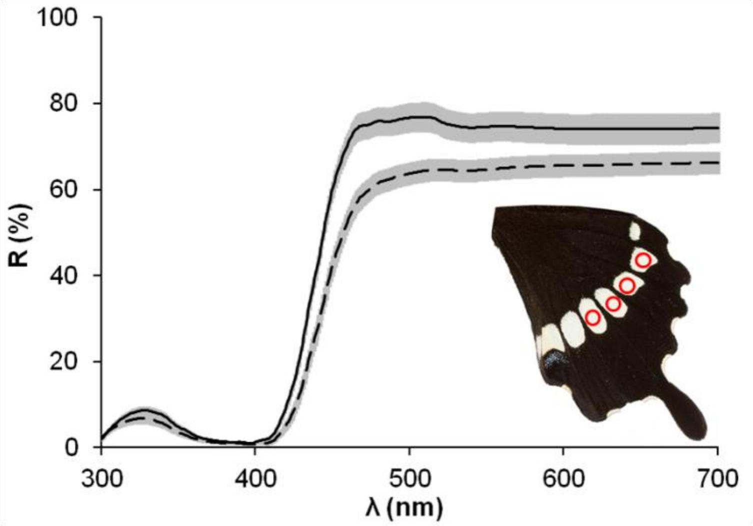

Figure 4.2. Sexual dimorphism in dorsal brightness of males (solid line) and non-mimetic females (dashed line) of *Papilio polytes*. Lines and shaded regions represent mean (n = 20 for both sexes) and standard deviation, respectively. Red circles on dorsal hindwing (image) indicate the locations where spectral measurements were taken for all individuals.

METHODS

Female Mate Choice

Two separate sets of experiments were conducted to investigate mate choice in the non-mimetic female form *cyrus* (hereafter *cyrus* female) and the mimetic female form *romulus* (hereafter *romulus* female). Full siblings were used in both sets of female mate choice experiments to control for the possibility of inbreeding avoidance.

Origin of Animals

Butterflies used were first-generation offspring of two females (one *romulus* and one *cyrus*) reared from wild larvae. Potted *Citrus* plants were placed outside a greenhouse (National Centre for Biological Sciences, Bangalore, India) to allow wild females to oviposit, and larvae were then collected and raised on *Citrus* plants until pupation. Upon eclosion, a *romulus* female was allowed to mate and subsequently provided with *Citrus* plants for egg-laying; her offspring was used to investigate mate choice in *romulus* females (among these offspring, four males and four females were used to start another generation; see Male Mate Choice). A *cyrus* female was similarly obtained from wild-collected larvae and mated with a male from a pure-breeding *cyrus* population established in the laboratory one year ago; her progeny was used to study mate choice in *cyrus* females.

Rearing Protocol

Larvae were reared in a climate-controlled greenhouse (temperature = 29.5±1.5°C; relative humidity = 55.8±5.1%) and provided with *Citrus* leaves in excess (either plants or cuttings) until pupation. Upon eclosion, male and female butterflies were kept in separate cages until they were used for mate choice assays. All adults were given *ad libitum* access to nectar (Birds Choice butterfly nectar) via artificial flowers from which they fed readily.

Male Colour Manipulation

Males were treated on the day before they were used in female mate choice trials. They were first temporarily immobilized by placing in a refrigerator (temperature ≈ 4°C) for one hour. For males assigned to the treatment group, some scales were gently removed from the white patches on the dorsal hindwings by dabbing lightly with a piece of blu-tack (Bostik). Control males were handled identically except that scales were removed from black areas on the ventral hindwings; ventral hindwings of males are not likely to be visible to females during male courtship (pers. obs.). Each male was assigned a unique number to aid in identification. They regained full mobility within 10 to 15 min at room temperature, and were used in mate choice experiments the following day.

Mate Choice Assay

Experiments were performed in a flight cage (dimensions: 1.8 × 1.8 × 1.8 m) inside the greenhouse. Since the greenhouse material absorbs UV, full-spectrum fluorescent tubes (300 to 700 nm; Narva Biovital) were positioned above the cage to compensate for the lack of UV within the greenhouse. Fresh nectar was provided via artificial flowers, and butterflies were observed to court and mate readily in the flight cage.

A total of 10 and nine trials involving *romulus* and *cyrus* females, respectively, were conducted to investigate female mate choice. Females used were 1 to 3 days old (mean±SD: *romulus* = 1.6±0.7; *cyrus* = 1.7±0.6), and males ranged from 2 to 6 days old (*romulus* = 3.2±0.9; *cyrus* = 3.3±0.9). Each trial began at 0900 hours when four virgin females (*romulus* or *cyrus*) and eight virgin males (four from treatment group and four from control group) were released into the cage, which was subsequently observed at every 10-min interval for the occurrence of mating. Each mating pair was immediately removed from the cage; the identity of the male was noted (i.e., treatment or control male), and one male from the other group was simultaneously removed from the cage to maintain the ratio of control to treatment males at 1:1. Observations were suspended at 1600 hours by isolating unmated females and resumed at 0900 hours the following day until all four females were mated. No individual was used more than once, and all butterflies were killed by freezing at the end of each trial. Male wing colouration was measured to assess the effect of treatment (see *Phenotypic measurements*).

Phenotypic measurements

Size

Forewing length was used as a proxy for body size, and was measured as the distance between forewing apex and thoracic insertion (to the nearest 0.01 mm) using digital calipers (Mitutoyo, Japan).

Colour

Hindwings of males were removed and mounted on white cardboard using white adhesive with the dorsal side facing up. Reflectance of the dorsal white band was measured using an Ocean Optics® (Dunedin, FL, USA) Jaz spectrometer with illumination provided by a PX-1 pulsed xenon lamp. Two optical fibers (both outfitted with a collimating lens) were used: the illuminating fiber was positioned at 90° to the wing surface and the collecting fiber was set at 45° to minimize glare. Ambient light was excluded by using a paper-box with black cloth, and all spectra were measured relative to a Spectralon® reflectance standard which reflects >96% of incident light. A total of eight spectral readings (four per hindwing) were taken for each male, and averaged reflectance values between 300 to 700 nm (interpolated to a step width of 1 nm) were used for further analysis (see *Visual modeling*).

Visual modeling

To evaluate the effect of male wing colour manipulation, I compared the dorsal reflectance of control and treatment males from a butterfly visual perspective using the *R* package *pavo* (Maia *et al.*, 2013). As nothing is known about the visual capabilities of *Papilio polytes*, data from the congener *Papilio xuthus* was used. Behavioural evidence indicates that *P. xuthus* has colour vision (Kinoshita *et al.*, 1999; Kinoshita & Arikawa, 2000; Kelber *et al.*, 2001), and its visual system is well characterized (reviewed by Arikawa 2003; Kinoshita & Arikawa 2014). In addition, evidence suggests that achromatic (i.e., brightness) and colour vision are distinct processes mediated by different sets of photoreceptors (Osorio *et al.*, 1999a; Osorio & Vorobyev, 2005; Koshitaka *et al.*, 2011; Kinoshita *et al.*, 2012; Hempel de Ibarra *et al.*, 2014), and hence perception of brightness and colour differences must be modeled separately.

Six classes of photoreceptors have been identified in *P. xuthus*, namely the ultraviolet (UV), violet (V), blue (B), green (G), red (R), and broadband (BB) receptors (Arikawa, 2003). The UV, V, B, G, and R receptors have distinct peak sensitivities at 360, 400, 460, 520, and 600 nm respectively, whereas the BB receptor has a very wide spectral sensitivity that spans from about 450 to 650 nm (Arikawa et al. 2003; see Figure 3.3 in Chapter 3). Despite having six photoreceptor classes, the colour vision of *P. xuthus* is tetrachromatic, involving the UV, B, G, and R receptors (Koshitaka *et al.*, 2008; refer to section on *Butterfly colour vision* in Chapter 3 for more details). In addition, brightness perception is likely to be mediated by the BB receptor (Koshitaka *et al.*, 2011).

To model perception from a butterfly visual perspective, the photon catch Q_i for each photoreceptor type i was calculated as a function of the photoreceptor spectral sensitivity (S_i), the irradiance spectrum incident on the colour patch (I), and the reflectance spectrum of the patch (R) over the visible spectrum (i.e., 300 to 700 nm):

$$Q_i = \int_{300}^{700} S_i(\lambda) I(\lambda) R(\lambda)\, d\lambda$$

Stoddard & Prum (2008) found that including both irradiance variation and colour constancy correction (by applying the von Kries transformation; see Vorobyev et al. 2001) leads to results that were effectively identical to those when assuming an idealized constant illumination, i.e. $I(\lambda) = 1$. Since behavioural evidence indicates that *P. xuthus* has colour constancy (Kinoshita & Arikawa, 2000), the above equation can be simplified to:

$$Q_i = \int_{300}^{700} S_i(\lambda) R(\lambda)\, d\lambda$$

According to Fechner's law, the intensity of the perceived stimulus is proportional to the logarithm of the actual stimulus intensity. Therefore, brightness perception mediated by the BB receptor (spectral sensitivity provided by K. Arikawa) is given by:

$$L_{BB} = \ln Q_{BB}$$

To assess whether scale removal also led to changes in chromatic aspects, I obtained measures of hue and chroma by mapping the reflectance spectra of control and treatment males in butterfly colour space. For an animal with tetrachromatic colour vision, any perceived colour can be mapped as a point in a tetrahedral space with its position in the tetrahedron determined by the relative stimulation of the four photoreceptor types (Goldsmith, 1990; Endler & Mielke, 2005; Stoddard & Prum, 2008). The four vertices of the tetrahedron correspond to the four different photoreceptors; for *Papilio* colour vision, they would be the UV, B, G, and R receptors (Figure 4.3). In this three-dimensional (3D) space, the achromatic point of equal receptor stimulation (i.e., white, black or gray) is placed at the origin (0,0,0) and any colour can be represented by its *X*, *Y*, and *Z* Cartesian coordinates.

Quantum catches of the four receptors involved in *Papilio* colour vision (i.e., UV, nB, dG and R) were first calculated as shown above. Spectral sensitivities of the photoreceptors were derived using the equation and parameters given in Koshitaka et al. (2008). These values were then normalized to sum to 1 to yield relative quantum catch q_i for receptor *i*:

$$q_i = \frac{Q_i}{\sum_i Q_i}$$

Each set of relative quantum catch values was then converted to a point in the tetrahedral colour space with *X*, *Y* and *Z* coordinates using the equations proposed by Endler & Mielke (2005):

$$X = \frac{(1 - 2q_B - q_G - q_{UV})}{2}\sqrt{\frac{3}{2}}$$

$$Y = \frac{3q_G + q_{UV} - 1}{2\sqrt{2}}$$

$$Z = q_{UV} - \frac{1}{4}$$

Each point in the tetrahedral colour space can also be expressed as a vector defined by its distance from and angle relative to the achromatic origin (see Figure 4.3). Points distributed along any straight line from the origin share the same hue but differ in chroma (i.e., saturation). Therefore, the length of the colour vector (i.e., distance from the origin to the point) is a measure of its chroma (Endler & Mielke, 2005):

$$r = \sqrt{X^2 + Y^2 + Z^2}$$

However, since the colour space is a tetrahedron and not a sphere, different hues vary in their potential maximum chroma r_{max}. Stoddard & Prum (2008) suggested that the chroma of a colour can be defined relative to the maximum chroma possible for its hue, giving the achieved chroma:

$$r_A = \frac{r}{r_{max}}$$

where $r_{max} = \frac{0.25}{\cos(\pi - \alpha_{\max})}$ and α_{max} is the largest of the angles formed between the colour vector and the four vertices of the colour space. I calculated values of r_{max} for all males as a measure of chroma.

Whereas the chroma of a colour is proportional to its vector length in colour space, hue is described by the angle of the vector relative to the origin. The difference in hue (i.e., hue disparity) between two colours A and B can be determined by the angle α between their colour vectors; if A and B are of similar hue, α will be close to zero (Stoddard & Prum, 2008). The cosine of the angle between colour vectors *A* and *B* is defined by the dot product divided by the product of their lengths:

$$\cos \propto = \frac{A \cdot B}{|A||B|}$$

where $A \cdot B = X_A X_B + Y_A Y_B + Z_A Z_B$. I calculated hue disparity between treatment and control males, and hue disparity within control males.

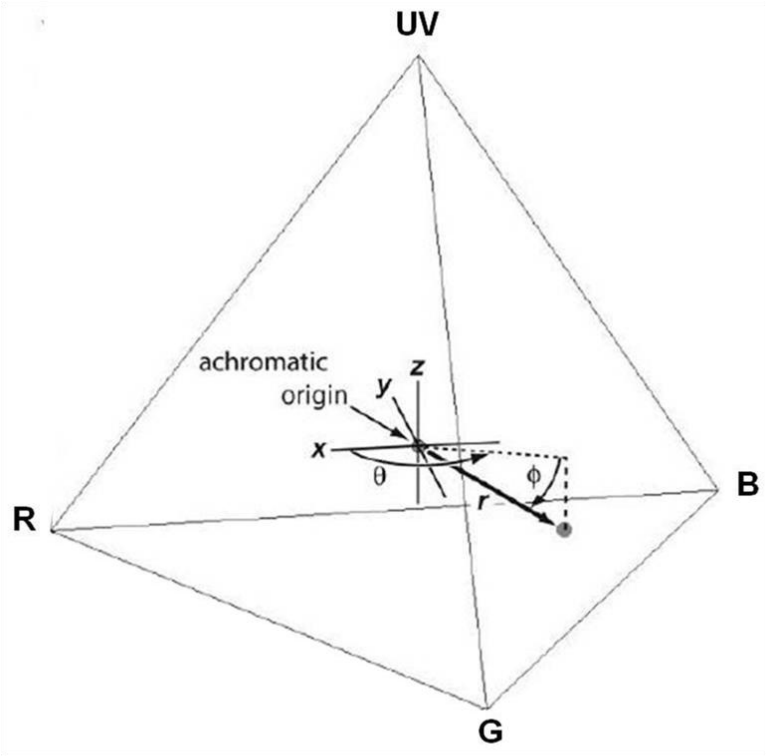

Figure 4.3. A tetrahedral colour space depicting *Papilio xuthus* colour vision. Each colour is a point in the tetrahedron with its position determined by the relative stimulation of the four photoreceptors (UV, B, G, and R). Colours which stimulate all receptors equally are placed at the achromatic origin. Each point in the colour space can be represented by its X, Y, and Z Cartesian coordinates. Alternatively, each colour can be expressed as a vector defined by its distance from the origin (r) and its angle relative to the origin (θ and ϕ). Vector length r is a measure of chroma, while angles θ and ϕ describe the hue. Taken and modified from (Stoddard & Prum, 2008).

Male Mate Choice

Origin of Animals

Four *romulus* females and four males (which were the siblings of individuals used in *romulus* mate choice trials) were allowed to mate, and the offspring of these butterflies were used in male mate choice trials. *Cyrus* females (and also males) were obtained from the laboratory pure-breeding *cyrus* population.

Rearing Protocol

Rearing protocol was the same as that described above in *Female Mate Choice*.

Mate Choice Assay

Trials to investigate male mate choice were conducted inside the same greenhouse with smaller flight cages (dimensions: 60 × 60 × 90 cm). One *romulus* female and one *cyrus* female were released into each cage about one hour before the start of the experiment, and allowed to feed on the nectar provided via artificial flowers. To control for mating receptivity, each pair of females was matched for age whenever possible and differed by no more than one day when it was not possible to get two females of the same age. The experiments commenced at 0900 hours by releasing one male into each cage. Cages were inspected for the occurrence of mating at every 10-min interval. When a mating pair was observed, the identity of the female (i.e., *cyrus* or *romulus*) was noted. Observations were suspended at 1600 hours by isolating the unmated male and resumed at 0900 hours the following day until mating occurred. Due to the unequal abundance of *romulus* and *cyrus* females obtained during the experimental period, unmated females in some trials were reused the following day. Males were used only once and no females were used more than twice. Females ranged from 1 to 3 days old (*romulus* = 1.6±0.8; *cyrus* = 1.7±0.8) and males were 3 to 5 days old (3.9±0.7). Butterflies were killed by freezing at the end of the experiments, and forewing length was measured using digital calipers as a measure of body size for all individuals.

Statistical Analyses

To assess the effect of wing colour manipulation, I compared measures of brightness (L_{BB}), chroma (r_A), and hue disparity (α) for control and treatment males involved in female mate choice experiments using Wilcoxon rank-sum tests. Chi-square tests were used to evaluate female preference, while independent t-tests or Wilcoxon rank-sum tests were used for size comparisons of control and treatment males, mated and unmated males, as well as females that mated with control versus treatment males. Male mate choice was similarly assessed using a chi-square test, while paired t-tests were used to determine whether there were size differences between *cyrus* and *romulus* females, and also between mated and unmated females. I used Shapiro-Wilk tests to check for normality of data, and conducted all statistical analyses in R (R Core Team, 2013) and SPSS (version 18). Values are presented as mean±SD.

RESULTS

Female Mate Choice

Male Colour Manipulation

Reflectance spectrometry showed that control males had higher dorsal reflectance than treatment males from approximately 450 to 700 nm, whereas treatment males reflected more short wavelengths (< 450 nm) than control males (Figure 4.4). Visual modeling using *Papilio* spectral sensitivities verified that experimental scale removal led to a reduction in male brightness, with control males being significantly brighter than treatment males in both sets of female mate choice experiments (Wilcoxon rank-sum test: *romulus* [control = 4.32±0.02 vs. treatment = 4.25±0.03], $W = 1541$, $N = 80$, $p < 0.001$; *cyrus* [control = 4.30±0.02 vs. treatment = 4.22±0.03], $W = 1285$, $N = 72$, $p < 0.001$; Figure 4.5A). Changes in chroma and hue from a butterfly visual perspective were also detected: treatment males had a significantly lower achieved chroma than control males (Wilcoxon rank-sum test: *romulus* [control = 90.42±1.03% vs. treatment = 78.49±7.66%], $W = 1585$, $N = 80$, $p < 0.001$; *cyrus* [control = 92.15±0.86% vs. treatment = 72.71±5.26%], $W = 1296$, $N = 72$, $p < 0.001$; Figure 4.5B), while hue disparity between treatment and control males was significantly greater than that within control males (Wilcoxon rank-sum test: *romulus* [between = 1.85±0.83° vs. within = 0.54±0.21°], $W = 38$, $N = 80$, $p < 0.001$; *cyrus* [between = 2.74±1.09° vs. within = 0.63±0.19°], $W = 0$, $N = 72$, $p < 0.001$; Figure 4.5C).

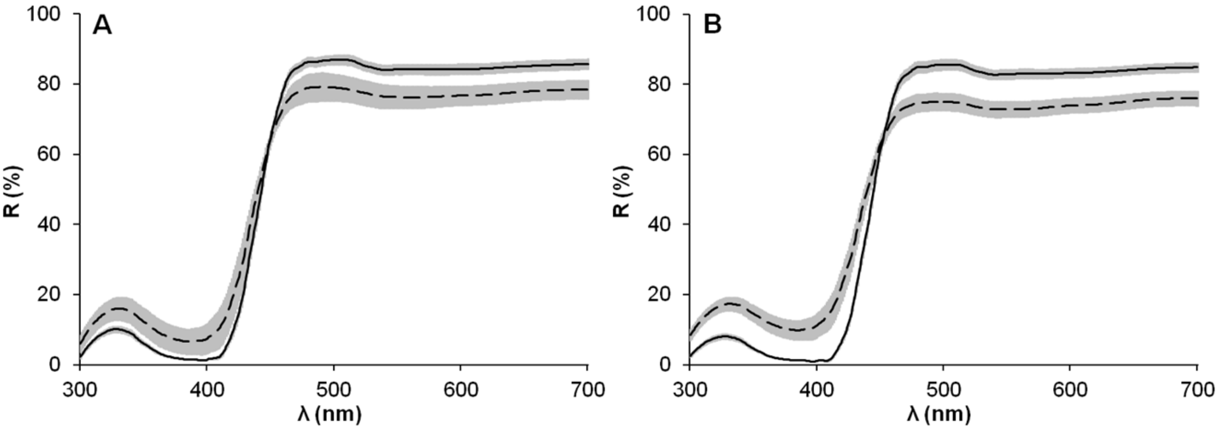

Figure 4.4. Dorsal reflectance of control (solid lines) and treatment (dashed lines) males used in *romulus* (A) and *cyrus* (B) female mate choice experiments. Lines and shaded regions represent mean values (A: $n = 40$ for both; B: $n = 36$ for both) and one standard deviation, respectively.

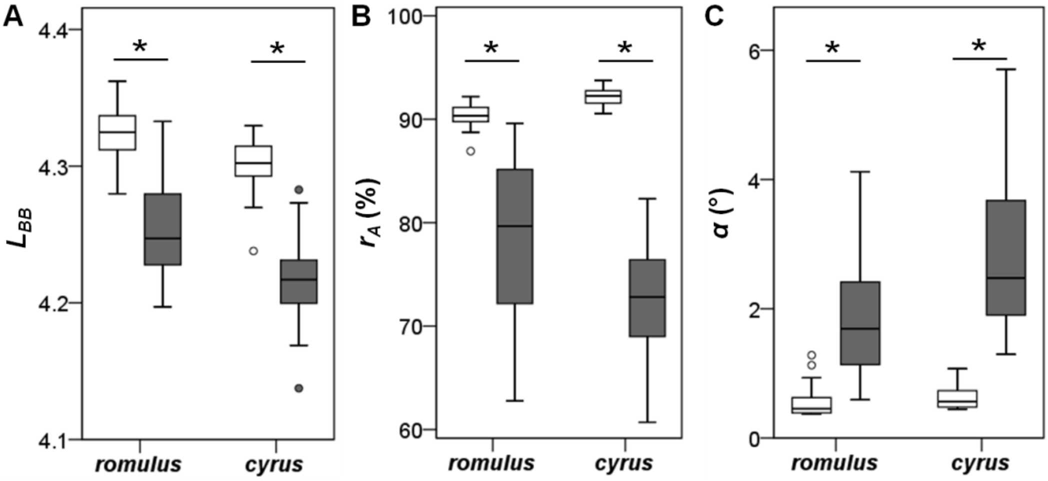

Figure 4.5. Wing colour manipulation of males used in female mate choice experiments from a *Papilio* visual perspective. (A) Dorsal brightness of control (white) and treatment (grey) males. (B) Achieved chroma of control (white) and treatment (grey) males. (C) Hue disparity within control males (white) and between treatment and control males (grey). Box plots show medians and quartiles (25 and 75%), and whiskers show data points up to 1.5 times the interquartile range. * $p < 0.001$

Female Preference

A total of 40 and 36 matings were observed in *romulus* and *cyrus* female mate choice trials respectively. No significant preference for either male phenotype was shown by *romulus* females (15 mated with control males and 25 mated with treatment males; $\chi^2_1 = 2.50$, $p = 0.11$). Among these 40 females, 31 (77%) mated on the first day of trial, five (13%) on the second day, and four (10%) on the third day. Similarly, *cyrus* females were equally likely to mate with either male phenotype (19 matings with control males and 17 matings with treatment males; $\chi^2_1 = 0.11$, $p = 0.74$). Out of these 36 matings, 25 (69%) occurred on the first day of trial, nine (25%) on the second day, and two (6%) on the third day.

Males that mated with *romulus* females did not differ in size from unmated males in those trials (mated = 42.15±2.98 mm vs. unmated = 42.18±3.34 mm; Wilcoxon rank-sum test: $W = 744$, $N = 80$, $p = 0.59$; Figure 4.6A). On the other hand, mated males were significantly larger than unmated males in trials involving *cyrus* females (mated = 41.41±2.26 mm vs. unmated = 39.60±2.95 mm; independent t test: $t_{70} = 2.91$, $p < 0.01$; Figure 4.6A). In addition, no significant size difference was found between females that mated with control males versus those that mated with treatment males for both sets of female mate choice experiments (independent t test: *romulus* [control = 43.66±3.54 mm vs. treatment = 45.09±2.96 mm], $t_{38} = -1.37$, $p = 0.18$; *cyrus* [control = 43.56±2.80 mm vs. treatment = 44.04±2.21 mm], $t_{34} = -0.57$, $p = 0.57$; Figure 4.6B). Lastly, there was no significant difference in size between control and treatment males in both sets of experiments (independent t test: *romulus* [control = 42.23±3.53 mm vs. treatment = 42.10±2.75 mm], $t_{78} = 0.19$, $p = 0.85$; *cyrus* [control = 40.69±2.86 mm vs. treatment = 40.32±2.69 mm], $t_{70} = 0.56$, $p = 0.58$).

As *cyrus* females preferred to mate with larger males (Figure 4.6A), I investigated whether male size was correlated with brightness of the dorsal white band, and found a significant positive relationship between the size of control males and their dorsal brightness (Pearson's correlation: $r = 0.29$, $p < 0.05$, $N = 76$; Figure 4.7).

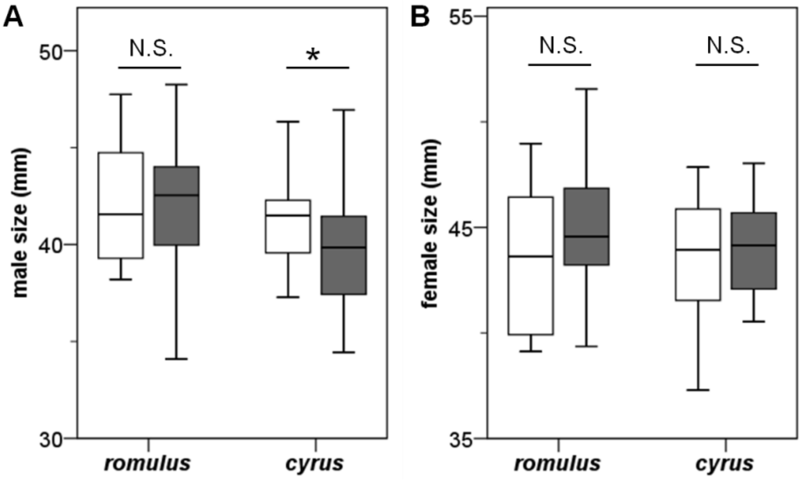

Figure 4.6. Size of mated (white) versus unmated (grey) males (A) and females that mated with control (white) versus treatment (grey) males (B) in *romulus* and *cyrus* female mate choice experiments. Box plots show medians and quartiles (25 and 75%), and whiskers show data points up to 1.5 times the interquartile range. N.S. and asterisk (*) denote $p > 0.05$ and $p < 0.01$, respectively.

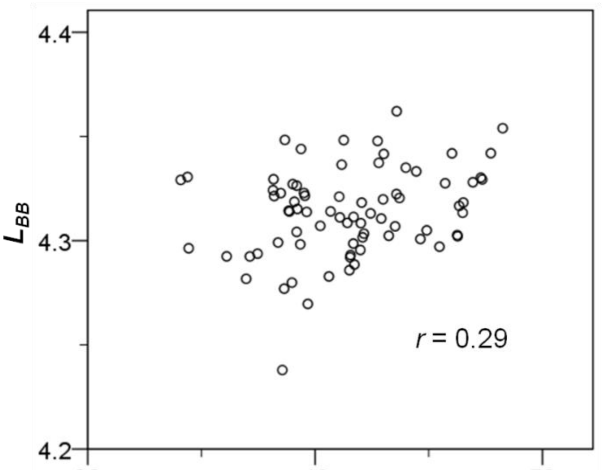

Figure 4.7. Relationship between brightness of dorsal white band and size of control males ($N = 76$) used in female mate choice experiments. Brightness was modeled from a *Papilio* visual perspective using spectral sensitivity of the BB photoreceptor. Treatment males were excluded from the analysis because their brightness had been reduced by scale removal.

Male Mate Choice

A total of 35 males mated in this set of experiments, with a significantly higher proportion of matings involving *romulus* females (24 males mated with *romulus* females and 11 mated with *cyrus* females; $\chi_1^2 = 4.83$, $p < 0.05$). All males mated by the second day of trials, and male preference for *romulus* females remained significant (i.e., $p < 0.05$) even when trials involving reused females (i.e., 8 *romulus* and 4 *cyrus* females) were excluded from the analysis.

Although *romulus* females were significantly larger than *cyrus* females (*romulus* = 44.12±2.58 mm vs. *cyrus* = 42.33 ± 2.25 mm; paired t test: $t_{34} = -4.53$, $p < 0.001$; Figure 4.8A), there was no significant difference in size between mated and unmated females (mated = 43.43±2.38 mm vs. unmated = 43.01±2.76 mm; paired t test: $t_{34} = -0.84$, $p = 0.41$; Figure 4.8B). Furthermore, males that mated with *romulus* females (40.72±2.33 mm) and those that mated with *cyrus* females (41.09±2.56 mm) did not differ in size (Wilcoxon rank-sum test: $W = 140$, $N = 35$, $p = 0.79$).

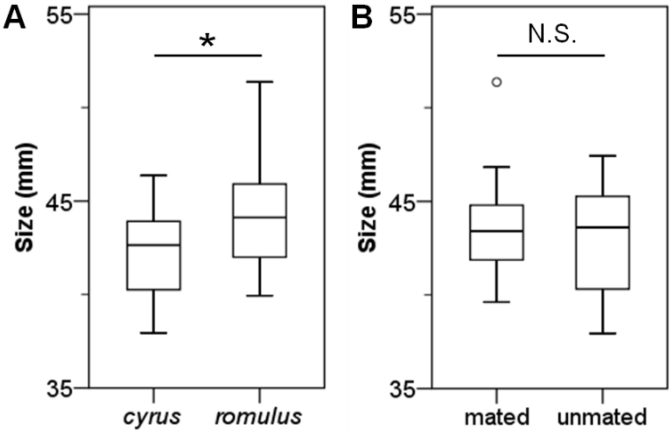

Figure 4.8. Size of *cyrus* versus *romulus* females (A) and mated versus unmated females (B) in male mate choice experiments. Box plots show medians and quartiles (25 and 75%), and whiskers show data points up to 1.5 times the interquartile range. Asterisk (*) and N.S denote $p < 0.001$ and $p > 0.05$, respectively.

As males seemed to show a preference for *romulus* females over *cyrus* females, I investigated whether mating rates observed during the two sets of female mate choice experiments were different. Although a comparable proportion of *romulus* and *cyrus* females mated on the first day across all trials (77% versus 69% respectively), an hourly breakdown revealed a huge difference in mating rate between the two female forms. At the end of the first hour across all trials (which began at 0900 hr), a total of 18 *romulus* females (45%) had mated whereas only four *cyrus* females (11%) had mated (Figure 4.9). Furthermore, 70% of *romulus* females (28 out of 40) mated within two hours of starting the trials, compared to only 31% of *cyrus* females (11 out of 36; Figure 4.9). In addition, I compared the sizes of females involved in the two sets of female mate choice experiments, and found no significant difference between them (*romulus* = 44.55±3.22 mm vs. *cyrus* = 43.78±2.52 mm; independent t test: $t_{74} = 1.15$, $p = 0.25$).

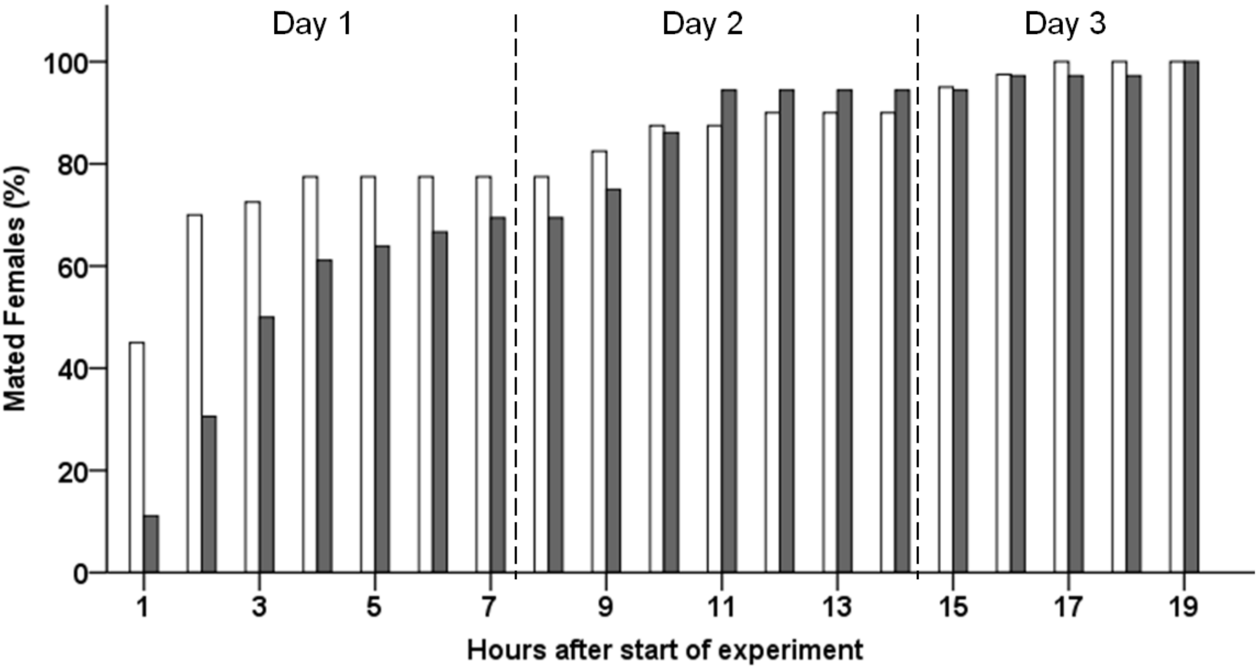

Figure 4.9. Mating rate of *romulus* (white) and *cyrus* (grey) female butterflies used in female mate choice experiments. Observations were taken from 0900 to 1600 hr for a total of seven hours per day. All *romulus* females ($n = 40$) and all *cyrus* females ($n = 36$) mated by 1200 hr and 1400 hr of day 3, respectively.

DISCUSSION

Female Mate Choice

Male wing colouration is known to influence female mate choice in several butterfly species, with females preferring to mate with males having brighter and/or more chromatic colours (Robertson & Monteiro, 2005; Davis *et al.*, 2007; Kemp, 2007, 2008b; Papke *et al.*, 2007; Morehouse & Rutowski, 2010; Rutowski & Rajyaguru, 2012). In this study, I artificially reduced the brightness of the white band found on the dorsal hindwings of male *Papilio polytes* butterflies, and conducted behavioural assays to investigate mate choice in the mimetic female form *romulus* as well as the non-mimetic female form *cyrus*. Visual modeling from a *Papilio* perspective showed that experimental scale removal to decrease male dorsal brightness achieved the intended effect, with control males being brighter than treatment males. However, both mimetic and non-mimetic females did not discriminate between males with artificially-reduced brightness and those with intact brightness (which were also more chromatic). These results suggest that male dorsal brightness does not affect female mate choice in this butterfly species.

Although females did not mate preferentially with brighter males, mated males in *cyrus* female mate choice trials were significantly larger than unmated males, indicating that females may prefer to mate with larger males. During copulation, male butterflies transfer an ejaculate (known as a spermatophore) that contains sperm and accessory substances to females. Nutrients found in the spermatophore can be utilized by females for egg production and somatic maintenance (Boggs & Glibert, 1979; Oberhauser, 1989, 1997; Svärd & Wiklund, 1991; Wiklund *et al.*, 1993; Kaitala & Wiklund, 1995; Karlsson, 1998; Stjernholm & Karlsson, 2000). Furthermore, in several species of Lepidoptera, the size of the spermatophore transferred during the first mating is positively correlated with male body size (Svärd & Wiklund, 1986; Wiklund & Kaitala, 1995; Bissoondath & Wiklund, 1996a; Hiroki & Obara, 1997; Hughes *et al.*, 2000; Ferkau & Fischer, 2006; Kemp *et al.*, 2008; Rajyaguru *et al.*, 2013). Since male-derived nutrients potentially contribute toward female reproductive output and larger males generally produce larger spermatophores, females may gain a greater direct benefit by mating with larger males. The fitness consequences of mating with larger versus smaller males can be explored in future work.

For holometabolous insects such as butterflies, adult size is heavily dependent on the amount and quality of nutrients acquired during larval development (Burghardt *et al.*, 2001; Fischer & Fiedler, 2001; Talloen *et al.*, 2004; Bauerfeind & Fischer, 2005; Boggs & Freeman, 2005; Kemp & Rutowski, 2007; Kemp, 2008a; Pegram *et al.*, 2013). Besides size, a reduced or low-quality larval diet could also have a detrimental effect on the synthesis of wing pigments during the pupal stage, thereby affecting the development and expression of adult wing colour traits (Burghardt *et al.*, 2001; Knüttel & Fiedler, 2001; Talloen *et al.*, 2004; Kemp *et al.*, 2006; Kemp & Rutowski, 2007; Kemp, 2008a; Pegram *et al.*, 2013). As both morphological traits (i.e., size and wing colouration) are dependent on larval nutrition, it is perhaps not surprising that I found a positive correlation between male size and dorsal brightness in this study. Larvae of the lycaenid butterfly *Polyommatus icarus* sequester flavonoids from their host plants to form wing pigments during the pupal stage, and flavonoid concentration in adult wings is positively correlated with measures of adult size (Burghardt *et al.*, 2001). Similarly, there is a positive relationship between the degree of wing melanization and adult wing size in the satyrine butterfly *Pararge aegeria* (Talloen *et al.*, 2004). Since size and dorsal brightness of male *Papilio polytes* butterflies appeared to be correlated traits, it is plausible that female preference for larger males could have led to the evolution of sexual dimorphism in dorsal brightness.

Male Mate Choice

Sexual selection by males has been proposed to explain the phenomenon of female polymorphism in female-limited mimetic butterflies (see Kunte 2009 for a review of hypotheses). In particular, Burns (1966) postulated that whereas mimetic female forms are favoured by natural selection, non-mimetic female forms are maintained in the population via male mate preference. When given a choice of *romulus* and *cyrus* females, males of *Papilio polytes* were found to mate preferentially with mimetic females, thereby refuting the hypothesis that persistence of non-mimetic females is due to male mate choice. On the other hand, Cook et al. (1994) observed that *Papilio dardanus* males approached the mimetic female form more often, and speculated that the less-preferred non-mimetic females have a selective advantage over mimetic females due to lower levels of male sexual harassment. Although fitness costs arising from male harassment have been documented in some animals (Magurran & Seghers, 1994; Clutton-Brock & Langley, 1997; Bateman *et al.*, 2006; Bots *et al.*, 2009; Gosden & Svensson, 2009; Takahashi & Watanabe, 2010), further investigation will be required to determine whether *romulus* females suffer from any fitness cost (e.g., reduced fecundity or longevity) associated with male preference.

As *romulus* females were larger in size than *cyrus* females in the paired trials, the higher mating success of *romulus* females could be due to males preferring to mate with larger females regardless of their phenotype. However, there was no difference in size between mated and unmated females across trials, indicating that male mate choice was independent of female size and was based on female phenotype. Furthermore, male preference for mimetic females was supported by the difference in mating rate observed during the two sets of female mate choice experiments: the number of *romulus* females mated by the end of the first hour was four times that of mated *cyrus* females. Since male butterflies and female butterflies used in these two sets of female mate choice experiments were of similar age, there should be no appreciable difference in their mating receptivity; in addition, *romulus* and *cyrus* females were of comparable size. Therefore, the discrepancy in mating rate was most likely caused by the greater sexual attractiveness of *romulus* females, as demonstrated by male mate choice assays.

Interestingly, male preference for the mimetic female phenotype might help to explain why a preference for larger males was detected in *cyrus* females but not *romulus* females. Males used in *romulus* female mate choice trials were observed to be extremely active: they chased each other around the flight cage vigorously and courted females very aggressively. Females were often courted by two or more males simultaneously, and this could potentially result in courtship interference (Wong & Candolin, 2005). Courtship interference due to male-male competition has been observed in several taxa, including fishes (Kangas & Lindström, 2001; Reichard *et al.*, 2004; Wong, 2004; Hibler & Houde, 2006), amphibians (Verrell, 1984; Hedlund, 1990; Sparreboom, 1996; Aragón, 2009), birds (Trail, 1985; Trail & Koutnik, 1986; Sæther *et al.*, 1999), and insects (Luan & Liu, 2012; Luan *et al.*, 2013). Under such circumstances, the assessment of male traits could be hampered or interrupted, and mating outcomes may not be a true reflection of female preference (e.g. Kangas & Lindström 2001; Reichard et al. 2005; Hibler & Houde 2006; McGhee et al. 2007). On the other hand, males involved in *cyrus* female mate choice assays appeared to be less active and engaged in courtship behaviour less often, such that females were usually courted by only one male at any time. In the absence of male-male competition and courtship interference, the outcome of mate choice trials involving *cyrus* females might be a more accurate representation of female preference, especially since females are likely to encounter males sequentially in the wild.

The faster mating rate and higher mating success of *romulus* females in female and male mate choice experiments indicated that males prefer to mate with *romulus* females. Since butterflies are known to use both visual cues and chemical signals (i.e., pheromones) for intraspecific communication (Vane-Wright & Boppre, 1993), *romulus* females might be more attractive to males visually and/or chemically. During preliminary trials when dried female specimens (i.e., no chemical cues available) were presented, male butterflies rarely courted these dead females regardless of their phenotype (personal observations), suggesting that female pheromones are paramount in eliciting male courtship behaviour in this species. Several butterfly behavioural studies have found that chemical cues are necessary for sexual discrimination and the release of male courtship behaviour (Wago, 1978; Boppré, 1984; Yamashita, 1995; Takanashi *et al.*, 2001; Morehouse & Rutowski, 2010). Chemical signals can also modulate the courtship and mating motivation of male butterflies (Andersson *et al.*, 2000, 2003; Schulz *et al.*, 2008; Estrada *et al.*, 2011). Ômura & Honda (2005) showed that males and *cyrus* females of *Papilio polytes* are sexually dimorphic in the chemical composition of volatile substances found on the wings and body, whereas the pheromone profile of mimetic females was not examined. The mating preference exhibited by males in this study suggests that the pheromone components of *romulus* females might differ from those of *cyrus* females in terms of chemical composition and/or concentration, rendering them more attractive to males.

Future Studies

Role of visual and chemical cues in butterfly mate choice

In this study, I investigated female mate choice in two female forms of *Papilio polytes* and also male mate preference given a choice of these two female forms; the other mimetic female form *stichius* was not included. It would be interesting to determine whether *stichius* females show any preference for brighter and/or larger males, and to examine the preference hierarchy shown by males toward these three female forms. In addition, to investigate whether a particular female form is more attractive to males because of its pheromone composition, the visual cues of two female forms can be standardized by blackening their dorsal wing surfaces before presenting them to males. No change in relative mating success would suggest that female pheromones are involved in male mate choice, and the pheromone profiles of the different female forms can be compared using gas chromatography-mass spectrometry (Ando & Yamakawa, 2011). Furthermore, to elucidate the relative importance of visual and chemical cues, the following female models can be prepared and offered to males: 1) unpainted and freshly killed; 2) unpainted and dried; 3) painted black and freshly killed; 4) painted black and dried. Male preference for the four different female models, measured in terms of courtship duration and number of copulation attempts, will then provide information on the relative importance of visual and chemical signals in male mate choice.

Role of female choice in evolution of female-limited mimicry

Sexual selection by females has been proposed to be one of the main drivers behind the evolution of female-limited mimicry in butterflies (Turner, 1978). However, empirical research to investigate whether female mate choice exerts stabilizing selection on male wing colouration is scant. Among the two published studies that tested this hypothesis in female-limited mimetic butterflies, the first study found that females of *Papilio glaucus* have a preference for males of the natural colour pattern over black-painted males (Krebs & West, 1988). On the other hand, wing colour manipulation did not affect male mating success in *Papilio polyxenes*, whereas male-male competition for mating territories seemed to play an important role in the maintenance of non-mimetic male colouration

(Lederhouse & Scriber, 1996). In the present study, I had performed a graded manipulation of male wing colouration; the visual signal, though modified in terms of brightness (and also saturation and hue), was retained in treatment males to allow the investigation, specifically, of whether females respond to signal variation displayed by males. Although this study found that females of *Papilio polytes* do not seem to be sensitive to signal variation among males, complete removal of the wing colour signal may result in a different outcome with respect to female mate choice (e.g., Kemp *et al.*, 2014), and would provide some insight to the role of female choice in the evolution of female-limited mimicry in butterflies.

Plasticity of male mate choice in female-polymorphic species

Although mate preference is often assumed to be a consistent trait, this may be maladaptive in female-polymorphic species (such as *Papilio polytes* and *P. dardanus*) because the relative frequencies of different female forms may show temporal and/or spatial fluctuations. In these polymorphic species, a flexible male mate preference based on prior encounters with females (i.e., learned frequency-dependent preference) may be more advantageous. Several damselfly species with female polymorphism have been found to exhibit frequency-dependent mate choice, with males preferring to mate with the more common female morph (Miller & Fincke 1999; van Gossum et al. 1999, 2001; Fincke et al. 2007; Gosden & Svensson 2009; Ting et al. 2009; Takahashi & Watanabe 2009; but see Rivera & Andrés 2001; Rivera & Sánchez-Guillén 2007; Hammers & van Gossum 2008; Xu & Fincke 2011). There is some evidence to suggest that male mate choice in female-polymorphic butterflies might also be frequency-dependent (Cook *et al.*, 1994), and that butterflies can learn to change their mate preference as a result of experience (Westerman *et al.*, 2012). Since *Papilio polytes* males used in this study were naïve (i.e., had no prior exposure to females), the findings of mate choice experiments showed that males have an innate preference for the *romulus* female form. Further investigation can be conducted to determine whether this innate male mate preference changes with experience and learning (i.e., whether it is plastic), as has been demonstrated for damselflies (Sánchez-Guillén *et al.*, 2013).

CHAPTER 5

ROLE OF SEXUAL SELECTION IN THE EVOLUTION OF FEMALE-LIMITED MIMETIC POLYMORPHISM

ABSTRACT

Several butterfly species exhibit female-limited mimetic polymorphism, whereby males are monomorphic and non-mimetic, while females occur in both mimetic and non-mimetic forms. Although Batesian mimicry is indisputably the evolutionary outcome of natural selection, sexual selection has been implicated in the evolution of female-limited mimetic polymorphism in two ways. First, female mate choice or male-male competition is suggested to exert stabilizing selection on male wing colouration; and second, male mate choice is postulated to maintain the non-mimetic female form in the population. However, these hypotheses have rarely been tested, and existing studies used surrogate measures which might not accurately reflect mate preference. Here I investigated the role of sexual selection in the evolution of female-limited mimetic polymorphism in the swallowtail butterfly *Papilio alphenor*. Behavioural experiments using lab-reared butterflies were conducted, and the occurrence of mating was used as a direct measure of mate preference. I found that females did not discriminate between males with the typical wing phenotype and those with altered colouration (i.e., painted black or blue), suggesting that female choice does not explain why males remain non-mimetic. In addition, males preferred to mate with mimetic than with non-mimetic females, implying that male choice is not involved in the maintenance of the non-mimetic female form. Furthermore, age and mating history were significant predictors of mating success in both male and female butterflies. I discussed the evolutionary significance of these findings and proposed potential future studies.

INTRODUCTION

Butterfly wing colours function as visual signals used in both intraspecific (e.g., conspecific recognition and mate choice) and interspecific (e.g., aposematism and mimicry) communication (Vane-Wright & Boppre, 1993; Kemp & Rutowski, 2011). Depending on the interactions between various selective regimes, wing colouration may represent a compromise between survival and reproductive success (Ellers & Boggs, 2003; Estrada & Jiggins, 2008; Morehouse & Rutowski, 2010). Furthermore, natural and sexual selection pressures may act differentially on the two sexes. For instance, in the pierid butterfly *Pieris napi*, dorsal wing melanization (a form of thermoregulatory adaptation) increases with increasing latitude in females but not males, suggesting that male wing colouration may be under constant sexual selection across latitudes (Tuomaala *et al.*, 2012). In addition, evolutionary forces may also differ between different morphs of the same sex. Female butterflies of the genus *Colias* exist in two forms: one form has yellow wings typical of the genus, while the other possesses white wings (‘alba’ females). Yellow females are more attractive to mate-locating conspecific males (Graham *et al.*, 1980; Nielsen & Watt, 2000; Kemp & Macedonia, 2007), whereas ‘alba’ females have a potential fecundity advantage as they allocate lesser resources to costly wing pigment synthesis (Graham *et al.*, 1980; Gilchrist & Rutowski, 1986). Therefore, natural and sexual selection pressures acting on butterfly wing colours may show intersexual as well as intrasexual differences.

Batesian mimicry, first described in butterflies more than a century ago (Bates, 1862), occurs when palatable species resemble distasteful ones to gain protection against predation. Interestingly, Batesian mimicry is limited to the female sex in several butterfly species (Kunte, 2008, 2009a). The evolution of female-limited mimicry is suggested to be due to higher predation risk faced by females (Ohsaki, 1995, 2005; Ide, 2006), which are potentially vulnerable to predators during oviposition at larval host plants and have a less effective escape flight than males due to their heavier abdomens (Srygley & Chai, 1990; Srygley & Dudley, 1993). As a result, female butterflies may benefit more from becoming mimetic. Stabilizing sexual selection on male wing colouration has also been hypothesized to play a role (Turner, 1978). For instance, in the female-limited mimetic butterfly *Papilio polyxenes*, males with dorsal wing colouration altered to resemble the mimetic pattern of females have lower success in establishing mating territories than males with the typical wing pattern (Lederhouse & Scriber, 1996). In addition, female butterflies of *Papilio glaucus* were found to have a preference for males with the natural wing colour pattern over those painted black to resemble the model *Battus philenor* (Krebs & West, 1988). Furthermore, mimetic females of the butterfly *Hypolimnas bolina* prefer to mate with non-mimetic males showing intact UV reflectance (Kemp, 2007). Hence, both intra- and intersexual selection might exert stabilizing selection on male wing colouration that prevents the evolution of Batesian mimicry in males.

Moreover, some female-limited mimetic butterfly species exhibit polymorphism, whereby the female exists in two or more distinct forms (Kunte, 2009a). This phenomenon is most prominent in the butterfly *Papilio dardanus*, where the male is monomorphic while the female occurs as at least nine distinct morphs, with most of them mimicking distasteful Danaini and Acraeini species (Nijhout, 2003; Thompson & Timmermans, 2014). In addition, several of these female-polymorphic species (e.g., *Papilio polytes* and *P. dardanus*) possess both mimetic and non-mimetic female forms (Clarke & Sheppard, 1972; Thompson & Timmermans, 2014). Due to their resemblance to unpalatable butterflies, mimetic females experience lower predation rates than non-mimetic females and are thus favoured by natural selection (Jeffords *et al.*, 1979; Ohsaki, 1995). The non-mimetic female form, on the other hand, has been hypothesized to be maintained by sexual selection via male mate choice (Burns 1966; but see Kunte 2009a). This hypothesis has been tested in only two species so far: male butterflies of *Papilio glaucus* did not seem to discriminate between mimetic and non-mimetic females (Pliske 1972; Platt et al. 1984; but see Burns 1966), whereas *Papilio dardanus* males were observed to approach mimetic females preferentially (Cook *et al.*, 1994). However, these studies used surrogate measures (e.g., spermatophore counts or number of approaches by males) which might not accurately represent male mate choice. Therefore, further investigation using a more direct measure of mate preference (i.e., mating success) is necessary to elucidate male choice for mimetic versus non-mimetic female forms in polymorphic butterflies.

In this study, I investigated the role of sexual selection in the evolution of female-limited mimetic polymorphism in the swallowtail butterfly *Papilio alphenor*. Behavioural experiments using laboratory-reared butterflies were conducted to answer two specific questions. First, does female mate choice exert stabilizing selection on male wing colouration? I have previously shown (see Chapter 4) that females of the closely related butterfly *Papilio polytes* do not discriminate between males with intact dorsal brightness and those with artificially reduced brightness, suggesting that females do not exert directional selection on male wing colouration. However, complete removal of the dorsal white band might interfere with mate recognition and hence affect male mating success. For instance, mating success of *Hypolimnas alimena* male butterflies was not affected by brightness variation in the dorsal blue band, but showed a reduction only when the band was completely obscured (Kemp *et al.*, 2014). Thus in the present study, gross manipulation of male wing colouration was applied to investigate its effect on male mating success in *P. alphenor*. Second, do males prefer to mate with mimetic or non-mimetic females? Although I have previously found that males of *P. polytes* prefer to mate with mimetic females (form *romulus*; see Chapter 4), the mimetic female form of *P. alphenor* has a distinctly different phenotype, and it is not known whether they would be similarly preferred by males. To test this hypothesis, the mating success of mimetic and non-mimetic females was measured. I discussed the evolutionary significance of my findings and proposed potential studies for future work.

METHODS

Study Species

Papilio alphenor was originally considered a subspecies of *Papilio polytes* found in the Philippines, but has been granted species status in light of recent phylogenetic evidence (Kunte *et al.*, manuscript in preparation). It is phenotypically very similar to *P. polytes*, except that males and non-mimetic females lack hindwing tails. Males are monomorphic and non-mimetic, whereas females occur in two forms: non-mimetic females (form *cyrus*) have a male-like wing pattern, while mimetic females (form *stichius*) are Batesian mimics of the toxic butterfly *Pachliopta aristolochiae* (Figure 5.1). The entire wing pattern is under the genetic control of a single autosomal locus (Kunte *et al.*, 2014), with the *stichius* allele dominant over the *cyrus* allele (Clarke & Sheppard, 1972).

Rearing Protocol

Butterflies used in this study were part of a laboratory population started from about 100 butterflies. The butterfly population was maintained in a climate-controlled room (temperature of approximately 28°C and relative humidity of 50-60%), where the experiment was also performed. Larvae were reared on *Citrus* plants and adults were given *ad libitum* access to nectar (Birds Choice butterfly nectar) via artificial flowers.

P. alphenor
♀ form *cyrus*
(non-mimetic)

P. alphenor
♂
(monomorphic)

P. alphenor
♀ form *stichius*
(mimetic)

Pachliopta aristolochiae
(model)

Figure 5.1. *Papilio alphenor* exhibits female-limited mimetic polymorphism, with two female forms (one non-mimetic and one mimetic).

Mate Choice Experiment

The experiment was carried out in a flight cage (dimensions: 1.8 × 1.8 × 1.8 m) provisioned with artificial nectar and larval hostplants. On each day, newly eclosed butterflies (if any) were released into the cage after their wings had fully spread. Prior to release, each butterfly was assigned a unique number on its ventral hindwings with a black marker to aid in identification. Observations began at 0900 hours when full-spectrum lights in the room were switched on. The cage was inspected at every 10-min interval for the occurrence of mating. When a mating pair was found, the identification numbers of the male and female butterflies were recorded. Observations were suspended at 1900 hours and resumed the following morning. These daily observations continued for a total of 60 days until all butterflies released into the cage had died. The total number of butterflies in the cage on any given day depended on the introduction of newly eclosed butterflies and mortality of older butterflies. A total of 138 males and 148 females (79 non-mimetic and 69 mimetic) were released into the cage during the experimental period.

Colour Manipulation and Phenotypic Measurements

Prior to release into the flight cage, wing colouration of some randomly chosen butterflies were altered by painting with a black or blue Sharpie marker. Specifically, all the white and red colour patches on both wing surfaces were completely painted over. Control butterflies were handled the same way but no paint was applied. The number of males painted blue and black were 20 and 27 respectively, while the number of females painted blue and black were 14 (10 non-mimetic and 4 mimetic) and 29 (14 non-mimetic and 15 mimetic) respectively. For all butterflies prior to release, wingspan (i.e., distance between forewing apex and base) was measured using vernier calipers to the nearest 0.5 mm, and body weight was measured with a weighing balance to the nearest mg.

Statistical Analyses

Generalized linear mixed-effects models (GLMM) were used to investigate the factors affecting mate choice in males and females separately, with *mating success* (mated or unmated) as the binary response variable. The following variables were included as fixed-effects in the models: *wing colour* (typical, blue, or black); *mimicry* (mimetic or non-mimetic; for females only); *wingspan*; *body weight*; *age*; and *mating history* (virgin or non-virgin). Only days on which at least one mating was observed were used in the analyses, and *day* was included as a random-effect variable in the models. Statistical analyses were conducted in R (R Core Team, 2013).

RESULTS

Matings were observed on 20 days, ranging from one to 10 matings per day, resulting in a total of 58 matings.

Male Mating Success

A total of 42 males mated, out of which 30 mated once, 10 mated twice and two mated four times. Wing colour was not a significant predictor of male mating success: males with the typical colouration were as likely to mate as males painted either black ($z = -0.71$, $p = 0.48$) or blue ($z = -0.49$, $p = 0.63$). On the other hand, age was a highly significant predictor of male mating success, with younger males having a higher probability of mating than older males ($z = -4.14$, $p < 0.001$). Mating history was also a significant predictor, with non-virgin males having a higher probability of mating than virgin males ($z = 2.64$, $p < 0.01$). Lastly, wingspan ($z = -0.11$, $p = 0.92$) and body weight ($z = 0.87$, $p = 0.39$) were not significant predictors of male mating success. Output of the GLMM is given in Table 5.1.

Table 5.1. Predictors of male mating success used in GLMM analysis

Variable	Estimate	SE	z
Wing colour			
black	−0.271	0.379	−0.714
blue	−0.204	0.419	−0.486
Age	−0.135	0.033	−4.138**
Mating history			
non-virgin	0.880	0.333	2.643*
Wingspan	−0.008	0.074	-0.106
Body weight	0.003	0.004	0.865

* $p < 0.01$; ** $p < 0.001$

Female Mating Success

A total of 54 females (19 non-mimetic and 35 mimetic) mated, among which 50 mated once and 4 mated twice. Mimicry was a significant predictor of female mating success, with mimetic females having a higher probability of mating than non-mimetic females ($z = 3.19$, $p < 0.01$). Age was also a highly significant predictor, with younger females having a higher probability of mating than older females ($z = -3.35$, $p < 0.001$). In addition, mating history was a significant predictor of female mating success, with virgin females having a higher probability of mating than non-virgin females ($z = -3.12$, $p < 0.01$). On the other hand, wing colour was not a significant predictor, with unpainted females being as likely to mate as females painted either black ($z = 0.49$, $p = 0.62$) or blue ($z = -1.37$, $p = 0.17$). Lastly, wingspan ($z = 1.02$, $p = 0.31$) and body weight ($z = -0.34$, $p = 0.74$) were not significant predictors of female mating success. Output of the GLMM is provided in Table 5.2.

Table 5.2. Predictors of female mating success used in GLMM analysis

Variable	Estimate	SE	*z*
Mimicry			
mimetic	0.930	0.292	3.190*
Age	−0.124	0.037	−3.347**
Mating history			
non-virgin	−1.721	0.551	−3.123*
Wing colour			
black	0.163	0.332	0.493
blue	−0.854	0.622	−1.372
Wingspan	0.088	0.086	1.023
Body weight	−0.001	0.004	−0.335

* $p < 0.01$; ** $p < 0.001$

DISCUSSION

Many butterfly species exhibit Batesian mimicry, which is the evolutionary outcome of natural selection (Ruxton *et al.*, 2004a). However, sexual selection has been hypothesized to play a role in the evolution of female-limited mimetic polymorphism in butterflies via two mechanisms: first, by exerting stabilizing selection on male wing colouration via female mate choice (Turner, 1978); and second, by exerting preferential selection for different female forms via male mate choice (Burns, 1966). In this study, I tested these hypotheses experimentally in the female-limited mimetic butterfly *Papilio alphenor*, using the occurrence of mating as a direct measure of mate preference. Females did not discriminate between males with the typical phenotype and those with altered wing colouration, whereas males preferred to mate with mimetic rather than with non-mimetic females. Age and mating history were also significant predictors of mating success in both male and female butterflies.

Male Mating Success

Wing colour manipulation did not affect male mating success in *Papilio alphenor*, with females showing no preference for males with the typical phenotype versus those with altered colouration. This suggests that female mate choice does not exert stabilizing selection on male wing colouration in this butterfly species. In contrast, females of *Papilio glaucus* exhibit a preference for males with the natural non-mimetic colour pattern over males painted black to resemble the mimetic females (Krebs & West, 1988). In another female-limited mimetic butterfly *Papilio polyxenes,* alteration of dorsal wing colour had no effect on male mating success; however, males with the typical phenotype were more successful during intrasexual contests for mating territories, suggesting that male-male competition plays an important role in the maintenance of non-mimetic male colouration (Lederhouse & Scriber, 1996). As males of *Papilio alphenor* are not known to be territorial, it seems unlikely that sexual selection, either in the form of female mate choice or male-male competition, could adequately explain why males are non-mimetic in this species. Consequently, female-limited mimicry has probably evolved mainly due to stronger natural selection on females, which experience higher predation risk and hence benefit more from mimicry (Ohsaki, 1995; Ide, 2006), in combination with the negative frequency-dependent advantage of Batesian mimicry (Lindström *et al.*, 1997).

Age was one of the significant predictors of male mating success, with younger males being more likely to mate as compared to older males. Other studies have found that female butterflies prefer to mate with younger males (Rutowski, 1985; Papke *et al.*, 2007; Karl & Fischer, 2013), and thus the higher mating probability of younger males found in this study may reflect female preference. Mating with older males led to a reduction in egg hatching success in the butterfly *Bicyclus anynana*, suggesting that older males may have reduced sperm viability (Karl & Fischer, 2013). Furthermore, since the probability of mating increases with age, females might prefer to mate with younger males because they are more likely to be virgins. Mating with virgin males may be beneficial to females because males transfer a larger spermatophore at first mating than at subsequent matings (Sims, 1979; Rutowski & Gilchrist, 1986; Svärd & Wiklund, 1986; Bissoondath & Wiklund, 1996a, 1996b; Hughes *et al.*, 2000; Ferkau & Fischer, 2006). In addition, duration of the first mating is generally shorter than that of subsequent matings (Sims, 1979; Rutowski & Gilchrist, 1986; Svärd & Wiklund, 1986; Rutowski *et al.*, 1987; Kaitala & Wiklund, 1995; Bissoondath & Wiklund, 1996a; Hughes *et al.*, 2000). Prolonged mating duration is detrimental to females since it reduces time available for oviposition and foraging, as well as increases predation risk (Almbro & Kullberg, 2009). Therefore, females may prefer to mate with younger males to maximize the quality of sperm and quantity of nutrients received from males, and to minimize time expenditure and predation risk associated with copulation.

Female butterflies may use both visual and chemical cues to differentiate males of different ages. Wing wear, which increases with activity and thus presumably increases with age, is used as a surrogate measure of butterfly age in many studies (Rutowski, 1985; Karlsson, 1987, 1994; Kemp, 2000, 2003, 2006a, 2006b; Bergström *et al.*, 2002; Välimäki & Kaitala, 2006; Papke *et al.*, 2007; Peixoto & Benson, 2008). Reduced wing reflectance as a result of wing wear has been shown to be indicative of male age (Kemp, 2006a) and might influence female choice, with females preferring to mate with fresher (and presumably younger) males (Rutowski, 1985; Papke *et al.*, 2007). Besides visual cues, chemical signals may also indicate age of male butterflies. In the butterfly *Bicyclus* *anynana*, quantitative variation in the male sex pheromone composition and cuticular lipid composition of different body parts were found to be representative of male age (Nieberding *et al.*, 2012; Heuskin *et al.*, 2014). Age-related differences in pheromone composition were also detected in male pierid butterflies (Kuwahara, 1979; Sappington & Taylor, 1990).

Mating history was also a significant predictor of male mating success. Interestingly, non-virgin males had higher mating success than virgin males, indicating that males that had previously mated were more likely to mate again as compared to their unmated counterparts. This implies that certain males possessed some particular trait(s) that consistently gave them a mating advantage over other males. One such possible trait is male courtship persistence, as there is evidence to suggest that courtship duration increases male mating success (Rutowski, 1985; Fischer, 2006; Papke *et al.*, 2007; Kehl *et al.*, 2014). However, no study has investigated whether courtship persistence differs among individuals and whether such inter-individual variation (if it exists) is consistent across time. In the butterfly *Bicyclus anynana*, there is some evidence to suggest that males with better flight ability (as defined by having larger forewings, a higher body mass and a higher fat content) are able to achieve consistently higher mating success (Kehl *et al.*, 2015). Besides behavioural traits, chemical cues may also show intraspecific differences which are stable over time. For instance, individual males of *Bicyclus anynana* were found to have specific pheromone blend characteristics that remain constant throughout their lifespan (i.e., several weeks), hence providing a reliable indicator of male identity and allowing the assessment of male quality (Nieberding *et al.*, 2012).

Females could gain a direct benefit from mating with larger males since male size is positively correlated with spermatophore size at first mating (Rutowski & Gilchrist, 1986; Svärd & Wiklund, 1986; Wiklund & Kaitala, 1995; Hiroki & Obara, 1997; Ferkau & Fischer, 2006; Vande Velde *et al.*, 2012; Rajyaguru *et al.*, 2013). However, male size (measured as forewing length and body weight at eclosion) was not a significant predictor of male mating success in *Papilio alphenor*. One possible explanation is that females might select against large males to reduce predation risk during copulation. When a pair of mating butterflies is disturbed, one of the mates takes off carrying its partner attached by the genitalia; in *Papilio* butterflies, it is the female who carries the male. As a result, mating with a larger and heavier male would hinder flight performance and increase predation risk (Almbro & Kullberg, 2009). Stabilizing rather than directional selection for male size has been found in *Colias eurytheme*, with females preferentially mating with males in the middle of the size range (Rutowski, 1985). Furthermore, it has been suggested that in weaponless animals such as butterflies, mating success is likely to be determined to a larger extent by intrinsic differences such as mating motivation, rather than by differences in physical attributes such as body size (Fischer *et al.*, 2008; but see Kehl *et al.*, 2015).

Female Mating Success

For butterfly species exhibiting female-limited mimetic polymorphism, sexual selection in the form of male mate choice has been suggested to be involved in the maintenance of the non-mimetic female form, while the mimetic female form is favoured by natural selection (Burns, 1966). In this study, mimetic females were found to have a higher mating success than non-mimetic females, suggesting that males preferred to mate with mimetic females. Thus, the mimetic female form is favoured by both natural and sexual selection in *Papilio alphenor*. Similar results were obtained for the closely related butterfly species *Papilio polytes*, where males also showed a mating preference for mimetic females (form *romulus*; see Chapter 4). Consequently, in regions where the models are present, the persistence of the non-mimetic female form is most likely to be an outcome of the negative frequency-dependent advantage of Batesian mimicry, rather than the result of sexual selection (Barrett, 1976; Kunte, 2009b). Furthermore, since the non-mimetic female phenotype is coded by a recessive allele (Clarke & Sheppard, 1972), it may be very difficult to eliminate the allele (and hence the phenotype) from the population because males and mimetic females could be carriers.

The exact reason why male *Papilio alphenor* butterflies may prefer to mate with mimetic females is currently unknown. As the two female phenotypes appear very different, mimetic females might be more attractive to males visually. However, the higher mating success of mimetic females was observed both among females with the typical wing colour pattern and among those with altered wing colouration, suggesting that male preference for the mimetic phenotype was not based on visual cues. Another potential explanation is chemical cues (i.e., pheromones), since butterflies are known to use both visual and chemical signals for intraspecific communication (Vane-Wright & Boppre, 1993). Sexual differences in pheromone composition have been detected in a number of butterfly species (Wago, 1978; Boppré, 1984; Ômura *et al.*, 2001; Takanashi *et al.*, 2001; Ômura & Honda, 2005). However, variation in chemical profile between different female forms in polymorphic species has not been investigated and should prove informative. Furthermore, it is not known whether mimetic females are generally more willing to mate than non-mimetic females. As mating carries costs such as increased predation risk and reduced time for foraging and oviposition, non-mimetic females may be more discriminative about mate selection to minimize the probability of having to remate (in the event that they mate with a low quality male). On the other hand, mimetic females may be less choosy since their resemblance to toxic butterflies offers them some degree of protection from predators, which would lower the costs of remating.

Similar to males, age was also a significant predictor of mating success in female butterflies, with younger females being more likely to mate than older females. Young females may be preferred by males because they have a longer reproductive lifespan than older females. Young females are also more likely to be virgins as compared to older females, and males may use age as a proxy to discriminate between mated and unmated females. Furthermore, younger females might produce offspring with higher fitness (Ducatez *et al.*, 2012a). Male preference for younger females could be mediated by visual and/or chemical cues. Older females presumably have greater wing wear than their younger counterparts, and the associated changes in wing reflectance could influence male mate choice (Rutowski, 1982). Variation in pheromone composition with age has been investigated mainly in male butterflies (Kuwahara, 1979; Sappington & Taylor, 1990; Nieberding *et al.*, 2012), and whether pheromone blend also changes with age in female butterflies is not well studied. In the butterfly *Bicyclus anynana*, cuticular lipid composition of different body parts changes with female age (Heuskin *et al.*, 2014) and might influence the behaviour of males, which court younger females more often and for longer duration (Kehl *et al.*, 2014).

Mating history was another significant predictor of female mating success, with females that had previously mated having lower mating success than virgin females, indicating that mated females were not likely to mate again. Rather than being the outcome of male preference, this finding probably reflects the reproductive strategy of females. In contrast to males, one or two matings are generally sufficient to maximize the reproductive success of females (Bateman, 1948; Trivers, 1972). Studies that examined spermatophore counts in wild-caught females found that *Papilio* butterflies are generally monandrous or mildly polyandrous, with one or two spermatophores found in most females (Burns, 1968; Ehrlich & Ehrlich, 1978; Sims, 1979; Watanabe & Nozato, 1986; Watanabe *et al.*, 1986). Furthermore, a single mating was shown to be sufficient to fertilize all the eggs of a *Papilio* female butterfly (Levin, 1973). A mated female should then maximize her reproductive success by spending her remaining time on oviposition and foraging (Wiklund, 1982). Mated *Papilio* females are able to reject courting males behaviourally (e.g., by adopting a mate refusal posture or flying away), while mated females of other butterfly species are known to discourage further courtship attempts chemically via male-transferred anti-aphrodisiacs (Andersson *et al.*, 2000, 2003; Schulz *et al.*, 2008; Estrada *et al.*, 2011).

Males might gain the most from their investment in nutrients and sperm by mating with large females since larger females typically have higher fecundity (Kimura & Tsubaki, 1986; Watanabe, 1988; Karlsson & Wickman, 1990; Hughes *et al.*, 2000; Bauerfeind & Fischer, 2005; Boggs & Freeman, 2005). However, no effect of female size on female mating success was detected in this study. Unlike females, males typically maximize their reproductive success by mating with as many females and as often as possible (Bateman, 1948; Trivers, 1972), hence they might be less discriminative and instead choose to mate with any willing female. In addition, wing colour manipulation did not affect female mating success in *Papilio alphenor*. It has been proposed that male butterflies use visual cues for long-range mate location, with chemical cues coming into play during sexual interactions at close proximity (Vane-Wright & Boppre, 1993). The size of the flight cage used in this study meant that females were likely to be readily conspicuous to males, thereby eliminating the need for mate location and the possible involvement of visual cues. Hence chemical cues, which were not altered, might play a more important role in male mating decisions. Alternatively, the visual signal that males respond to might had remained largely unchanged by the colour manipulation performed here; for instance, male butterflies might be using the blackness of the wing to locate potential mates (see Yamashita, 1995). As a result, painting the white and red wing patches of females blue or black would have a negligible impact on mate recognition and mate choice by males.

Future Studies

Role of chemical cues in sexual signaling

Although both visual and chemical cues may be used simultaneously in butterfly mate choice (Costanzo & Monteiro, 2007; Papke *et al.*, 2007), the relative importance of chemical cues during sexual interactions may be greater in mimetic species because visual cues become less reliable for mate recognition. As manipulation of female wing colouration did not affect male preference for mimetic females, it is likely that the greater sexual attractiveness of mimetic females is due to non-visual cues, implying that chemical differences exist between mimetic and non-mimetic females. Pheromone profiles of the different female forms can be determined and compared using gas chromatography-mass spectrometry (Ando & Yamakawa, 2011). Subsequently, the role of female pheromones in male mate choice should be investigated, for instance, by manipulating the ability of males to perceive chemical signals using their antenna (e.g., Costanzo & Monteiro, 2007). In addition, whether chemical cues can be used as signals of butterfly age and identity, as shown for *Bicyclus anynana* (Nieberding *et al.*, 2012), ought to be studied to give a better understanding of the potential signal content of chemical cues.

Effect of courtship persistence on male mating success

In weaponless animals such as butterflies, mating success has been suggested to be determined largely by intrinsic differences such as mating motivation, rather than by physical differences (Fischer *et al.*, 2008). Evidence from several studies indicate that courtship persistence, which is a measure of mating motivation, increases male mating success (Rutowski, 1985; Fischer, 2006; Papke *et al.*, 2007; Kehl *et al.*, 2014). Future research can investigate the effect of courtship duration on male mating success by conducting behavioural assays. Furthermore, in *Bicyclus anynana*, older males display higher levels of aggression and persistence during courtship, resulting in higher mating success than younger males (Fischer *et al.*, 2008; Nieberding *et al.*, 2012; Kehl *et al.*, 2014). Thus, the influence of age on male courtship duration needs to be considered. Moreover, whether courtship persistence differs among individuals and whether such inter-individual variation (if it exists) is consistent across time should also be examined. For instance, the butterfly *Pieris brassicae* exhibits intraspecific variation in mobility and the mobility-related traits of an individual were found to be consistent across time, potentially making some individuals better dispersers than others (Ducatez *et al.*, 2012b).

CHAPTER 6

GENERAL DISCUSSION

The diverse wing colours of butterflies serve various adaptive functions such as thermoregulation, camouflage, aposematism, mimicry, species recognition, and mate choice (Vane-Wright & Boppre, 1993; Ingram, 2009; Kemp & Rutowski, 2011). As a result of their multifunctional nature, butterfly wing colouration is likely to experience two or more selective forces simultaneously, and may represent a compromise between survival and reproductive success (e.g., Ellers & Boggs, 2003). This thesis examines how natural and sexual selection influence the evolution of Batesian mimicry and sexual dichromatism in butterflies. Using reflectance spectrometry and visual modelling, I quantified and analyzed butterfly wing colours from the visual perspectives of conspecifics and predators. I also conducted behavioural experiments to test hypotheses regarding the role of mate choice in the evolution of sexual dichromatism and female-limited mimetic polymorphism in butterflies.

Natural selection has led to the evolution of Müllerian mimicry among unpalatable species, as well as the evolution of Batesian mimicry in palatable ones (Ruxton *et al.*, 2004a). Although both types of mimicry occur in butterflies, they have rarely been assessed in an ecologically meaningful manner (but see Llaurens et al. 2014). Therefore, I first analyzed mimetic resemblance in butterfly mimicry rings of the Western Ghats, India, from the visual perspective of avian predators, which are presumably the intended recipients of butterfly mimicry (Chapter 2). Wing colour discriminability between Batesian mimics and their unpalatable models was quantified using the receptor noise model which took into account the visual sensitivities and photoreceptor abundances of insectivorous birds (Vorobyev & Osorio, 1998). I found that females of sexually monomorphic mimetic species were better Batesian mimics than their male counterparts, suggesting stronger natural selection on females and/or antagonistic sexual selection on males. In addition, female-limited and sexually monomorphic mimetic species were equally good mimics, implying that intersexual genetic correlations did not constrain the evolution of Batesian mimicry in female-limited mimics. Furthermore, mimetic resemblance was higher on the ventral wing surface than on the dorsal surface, suggesting stronger natural selection on the ventral surface and/or the presence of antagonistic sexual selection acting on dorsal colouration. Thus, natural and sexual selection may act differently on the two sexes and on the two wing surfaces to affect mimetic resemblance in a sex- and wing surface-specific manner.

Although sexual dimorphism is often treated as a hallmark of sexual selection, natural selection can also result in the evolution of sexual dimorphism (Stuart-Fox & Moussalli, 2007; Kunte, 2008). Using a group of swallowtail butterflies (subgenus *Menelaides*), I showed that sexual dimorphism in wing colour patterns was closely associated with female-limited Batesian mimicry, indicating that natural selection was the main driver of sexual dichromatism in these butterflies (Chapter 3). In addition, reflectance spectrometry measurements revealed that the white hindwing patches of non-mimetic *Papilio* butterflies absorbed UV strongly, whereas those of mimetic butterflies and toxic butterflies were UV-reflective. Next, by plotting these spectral readings in avian tetrahedral colour space, I discovered that wing colouration of mimetic butterflies overlapped with those of toxic butterflies, while wing reflectance of non-mimetic butterflies did not overlap with those of mimetic or toxic butterflies. Therefore, mimetic butterflies exhibited adaptive wing colour changes (from UV-absorbing to UV-reflecting) which were not apparent to the human eye. Furthermore, butterfly species with sexually monomorphic wing patterns also displayed sexual dichromatism in brightness from the visual perspectives of both butterfly conspecifics and avian predators. The stronger visual contrast found in males suggests that these wing colour traits may play a role in sexual signaling, or that females may be under stronger natural selection to be less conspicuous. Together, these results highlighted the importance of analyzing colour traits using the visual perception of relevant receivers to avoid the biases and limitations of human vision.

The brighter white hindwing patches of male *Papilio* butterflies might have evolved under directional sexual selection by female mate choice. I tested this hypothesis using the butterfly *Papilio polytes*, which shows female-limited mimetic polymorphism (Chapter 4). By artificially manipulating male dorsal brightness and conducting behavioural experiments, I measured the mating success of males with intact brightness and those with reduced brightness. Both non-mimetic (form *cyrus*) and mimetic (form *romulus*) females showed no mating preference for either type of males, which suggests that the brightness of the white dorsal band does not affect female mate choice. However, males that successfully mated with non-mimetic females were larger than unmated males, implying that male size might influence female mating decisions. Furthermore, there was a positive correlation between male size and male dorsal brightness, suggesting that female preference for larger males might have caused indirect selection for brighter dorsal reflectance in males. In addition, adaptive wing pattern changes associated with the evolution of Batesian mimicry in female butterflies might have implications for sexual selection, i.e. mimetic females may appear less attractive to males as compared to non-mimetic females. To investigate this, I offered male butterflies a choice of non-mimetic and mimetic females, and noted the mating success of each female form. Males mated preferentially with mimetic females, indicating that the mimetic female form is favoured by both natural and sexual selection. Consequently, the persistence of the non-mimetic female form is most likely due to negative frequency-dependent selection associated with Batesian mimicry, and/or the recessive nature of the *cyrus* allele.

Sexual selection has been postulated to play a role in the evolution of female-limited mimetic polymorphism (Burns, 1966; Turner, 1978). I investigated this hypothesis experimentally using the swallowtail butterfly *Papilio alphenor* (Chapter 5). To examine whether female mate choice exerts stabilizing sexual selection on male wing colouration, the white wing patches of some male butterflies were completely painted over, and behavioural experiments were performed. Females did not discriminate between males with the typical phenotype and those with manipulated wing colours, implying that male colouration is not under stabilizing selection by female mate choice. Hence, sexual selection does not appear to explain why males are non-mimetic in this butterfly species. In addition, male mate choice has been proposed to maintain the non-mimetic female form in female-limited mimetic species with both mimetic and non-mimetic female forms. However, consistent with results obtained in the previous chapter, I found that males preferred to mate with mimetic females (form *stichius*) than with non-mimetic ones (form *cyrus*). This strongly suggests that persistence of the non-mimetic female form in the population is not due to sexual selection via male choice. Furthermore, the greater sexual attractiveness of mimetic females might be due to chemical rather than visual differences between the two female forms, suggesting that chemical cues may play an important role during sexual interactions in mimetic butterfly species. Lastly, age and mating history significantly affected mating success in both male and female butterflies.

Overall, the results from this thesis contribute significantly to our understanding of how predation pressure and mate choice may influence the evolution of Batesian mimicry and sexual dichromatism in butterflies. I have demonstrated that the evolution of butterfly wing colours is likely to be influenced simultaneously by both natural and sexual selection pressures. More importantly, my findings suggest that the strength and direction of selection pressures may show inter- and intrasexual differences, and may also vary between the two wing surfaces. Furthermore, it is crucial to assess colour signals in a more ecologically relevant way by considering the visual capabilities of the intended receivers. Below are some suggestions of promising avenues for future research on butterfly wing colours.

Future Directions

Multiple Predators

In this study, birds were presumed to be the main selective agents of protective colouration in butterflies. However, other animals may also exert strong predatory pressure on butterflies, and thus influence the evolution of butterfly wing colours. For instance, lizards are known to feed on butterflies (e.g., Lyytinen et al. 2003; Vlieger & Brakefield 2007). Diurnal lizards have tetrachromatic colour vision (Loew *et al.*, 2002; Bowmaker *et al.*, 2005; Macedonia *et al.*, 2009; Fleishman *et al.*, 2011), and have been shown to be sensitive to prey colouration (Tseng *et al.*, 2014). More importantly, evidence suggest that lizards are able to discriminate between palatable and unpalatable butterflies, implying that they could act as selective agents for the evolution of aposematism and mimicry in butterflies (Boyden, 1976; Ehrlich & Ehrlich, 1982; Larsen, 1992, 2006). Besides vertebrates such as birds and lizards, carnivorous invertebrates may also be important predators of butterflies. Dragonflies have been observed to predate substantially on butterflies (Alonso-Mejia & Marquez, 1994; Sang & Teder, 2011; Tiitsaar *et al.*, 2013). Since dragonflies have colour vision with visual sensitivity ranging from UV to ~700 nm (reviewed by Bybee et al. 2012a), they could potentially exert selective pressure on butterfly wing colouration. Spiders are also known to feed on lepidopterans (Brown, 1984; Vasconcellos-Neto & Lewinsohn, 1984; Rota & Wagner, 2006; Sourakov, 2013). In particular, jumping spiders (family Salticidae) possess trichromatic colour vision (Zurek *et al.*, 2015) and are responsive to prey colour, exhibiting colour biases when foraging (Taylor *et al.*, 2014). Hence, they may drive the evolution of protective colour patterns in small invertebrate prey such as lycaenid butterflies (e.g., Sourakov 2013). Furthermore, predators with monochromatic vision (i.e., colour-blind), such as praying mantids (Sontag, 1971; Rossel, 1979), may also impose selection pressure on wing patterns of butterflies (e.g., Prudic et al. 2015). Therefore, butterfly wing colours are likely to be under selection by a diverse community of both vertebrate and invertebrate predators. The presence of multiple predatory species has important implications for the evolution of prey colouration because different predators may have different visual acuities (Endler & Mappes, 2004; Willink *et al.*, 2014; Dreher *et al.*, 2015; Prudic *et al.*, 2015). Thus, knowledge of the visual capability, cognitive ability, and behavioural ecology of the various predators will be necessary to gain a more holistic and accurate understanding of the suite of selective pressures acting on butterfly wing colours.

Butterfly Vision

Butterfly wing colours which function as sexual signals during intraspecific interactions must be assessed from the visual perspective of conspecifics. However, the visual capabilities of butterflies are less well studied as compared to that of birds, and spectral sensitivities are known for only a small handful of butterfly species (see Frentiu & Briscoe 2008). Furthermore, unlike avian vision, butterfly visual sensitivities appear to be less evolutionarily conserved (Frentiu & Briscoe, 2008; Osorio & Vorobyev, 2008). The ancestral butterfly eye contains visual pigments with peak absorbance in the ultraviolet, blue, and long-wavelength part of the visible spectrum (Briscoe, 2008). Diversification of the ancestral trichromatic system was subsequently achieved by lineage-specific opsin gene duplications (Briscoe, 2001; Arikawa *et al.*, 2005; Sison-Mangus *et al.*, 2006; Frentiu *et al.*, 2007; Awata *et al.*, 2009; Briscoe *et al.*, 2010). Further modification of photoreceptor spectral sensitivities was then accomplished by the presence of filtering pigments, which act as long-pass spectral filters (Arikawa *et al.*, 1999a, 1999b, 2009; Wakakuwa *et al.*, 2004; Zaccardi, 2006; Stavenga & Arikawa, 2011; Ogawa *et al.*, 2013). In addition, studies have found that some butterfly species have sexually dimorphic eyes due to sex-specific expression of visual and/or filtering pigments (Bernard &

Remington, 1991; Arikawa *et al.*, 2005; Sison-Mangus *et al.*, 2006; Everett *et al.*, 2012; Ogawa *et al.*, 2012, 2013), which imply that wavelength discrimination differs between the two sexes. For instance, male *Pieris rapae crucivora* butterflies possess enhanced spectral discrimination at short wavelengths due to the presence of a fluorescent filtering pigment that is not found in female eyes (Arikawa *et al.*, 2005). Furthermore, this sexual dimorphism is hypothesized to have evolved to improve male discrimination of female and male conspecifics, whose wing colours show sexual differences in UV reflectance (Arikawa *et al.*, 2005). The co-evolution of butterfly vision and wing colouration has important implications particularly for mimetic species. *Heliconius* butterflies have an additional UV photoreceptor as well as UV-reflective yellow wing pigments that are not found in related genera (Briscoe *et al.*, 2010). Visual modelling showed that the co-occurrence of enhanced UV vision and UV-reflecting wing pigments may allow *Heliconius* butterflies private intraspecific communication in the presence of co-mimics and predators (Bybee *et al.*, 2012b). These findings suggest that there may be correlated evolution between butterfly photoreceptors and wing colours involved in sexual signalling and/or predator avoidance. Therefore, more research on the visual systems of butterflies will be required to provide a greater insight on how natural and sexual selection pressures interact to shape the evolution of butterfly wing colours.

Signal Content

The bright conspicuous wing patterns of many unpalatable butterfly species act as aposematic signals that warn predators of their unprofitability (Ruxton *et al.*, 2004a). Besides being qualitative indicators of unpalatability, recent work suggest that warning colours may also function as quantitatively honest signals of toxicity (reviewed by Summers et al. 2015). Theoretical studies show that honest signaling in aposematic colouration is likely to evolve under certain conditions (Blount *et al.*, 2009; Franks *et al.*, 2009; Lee *et al.*, 2011; Holen & Svennungsen, 2012; Speed & Franks, 2014). This is supported by empirical research in Neotropical dendrobatid frogs, where visual conspicuousness has been found to be positively correlated with toxicity levels across populations and species (Summers & Clough 2001; Santos & Cannatella 2011; Maan & Cummings 2012; but see Darst et al. 2006; Wang 2011). Positive correlations between signal intensity and strength of defence have also been found in insects such as paper wasps (Vidal-Cordero *et al.*, 2012) and ladybird beetles (Bezzerides et al. 2007; Arenas et al. 2015; but see Blount et al. 2012). However, to the best of my knowledge, no published studies has investigated whether wing colours of aposematic butterflies function as quantitatively honest signals of toxicity, even though variable levels of chemical defence are known to exist within and among species (Brower *et al.*, 1984; Cohen, 1985; Trigo & Brown, 1990; Ritland, 1994; Bowers & Williams, 1995; Moranz & Brower, 1998; Fordyce *et al.*, 2005; Fordyce & Nice, 2008). Potential mechanisms for honest signaling should also be evaluated; for instance, variation in signal and defence may involve a trade-off in resource allocation, which can be investigated experimentally by varying nutrient availability during larval development (e.g., Blount et al. 2012). Furthermore, sexual selection may influence the evolution of aposematic colouration, as demonstrated in dendrobatid frogs (Maan & Cummings, 2009; Crothers *et al.*, 2011; Crothers & Cummings, 2013) and wood tiger moths (Nokelainen *et al.*, 2012; Gordon *et al.*, 2015). The interplay between natural and sexual selection in aposematic butterfly species has so far been examined only in the genus *Heliconius* (Jiggins *et al.*, 2001, 2004; Naisbit *et al.*, 2001; Mavárez *et al.*, 2006; Kronforst *et al.*, 2007; Munoz *et al.*, 2010; Merrill *et al.*, 2011), and more work on other butterfly species will certainly be beneficial. In addition, wing colour traits that potentially function as honest signals of mate quality and/or unpalatability are expected to be strongly condition dependent (Kemp & Rutowski, 2007; Kemp, 2008a; Pegram *et al.*, 2013), and more research is needed to elucidate the fitness consequences of these genotype × environment interactions. Similarly, although butterfly mate choice based on wing colouration is known to occur (Robertson & Monteiro, 2005; Kemp, 2007, 2008b; Papke *et al.*, 2007; Morehouse & Rutowski, 2010; Rutowski & Rajyaguru, 2012), the direct and/or genetic benefits of such mating preferences remain largely unknown (but see Kemp et al. 2008; Rajyaguru et al. 2013). Hence, the signal content of butterfly wing colours used in intra- and interspecific communication, as well as the associated mechanisms and fitness consequences, are important future avenues of research.

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APPENDIX

Table 1. Intersexual relative discriminability of butterfly models from an avian UVS and VS visual perspective

	UVS			VS		
Butterfly species	a	b	c	a	b	c
Danaus c. chrysippus[1]	0.09	-0.09	-	0.15	-0.05	-
	0.02	0.08	-	0.06	0.12	-
Delias eucharis[2]	5.58	-	-	2.49	-	-
	1.68	0.37	-0.17	0.54	0.07	-0.17
Pachliopta hector[3]	-0.03	6.23	-	-0.04	5.83	-
	0.05	1.50	-	0.03	1.81	-
Pachliopta a. aristolochiae[3]	0.08	1.88	-	0.09	1.55	-
	0.09	-0.02	-	0.05	-0.04	-
Pachliopta pandiyana[3]	-0.65	-0.81	-	-0.64	-0.42	-
	0.12	0.55	-	0.10	0.54	-
Danaus g. genutia[1]	-0.15	0.30	-	-0.04	0.20	-
	-	-	-	-	-	-
Cethosia mahratta[1]	0.19	4.35	-	0.17	2.64	-
	-	-	-	-	-	-
Euploea c. core[4]	-0.11	-0.25	-	-0.11	-0.16	-
	-0.10	-0.17	-	-0.04	-0.11	-
Euploea klugii kollari[4]	0.59	-0.10	-	0.60	-0.07	-
	0.42	0.57	-	0.66	0.38	-
Euploea sylvester coreta[4]	0.03	-0.19	-	0.15	-0.19	-
	0.03	-0.08	-	0.05	-0.08	-
Tirumala limniace exoticus[4]	-0.06	-0.58	-	0.07	-0.39	-
	-0.44	-0.39	-	-0.21	-0.29	-
Tirumala septentrionis dravidarum[4]	0.00	0.53	-	-0.06	0.52	-
	-0.09	-0.10	-	-0.06	-0.21	-
Parantica a. aglea[4]	0.09	-0.28	-	0.06	-0.25	-
	1.12	-0.36	-	0.78	-0.31	-
Parantica nilgiriensis[4]	1.63	0.34	-	0.21	0.58	-
	-0.30	0.98	-	-0.25	1.34	-

For each species, the top and bottom rows represent intersexual relative discriminability of dorsal and ventral wing colouration, respectively. Letters (a-c) represent different wing colour patches (see below).

[1] a: white; b: orange

[2] a: white; b: yellow; c: red

[3] a: white; b: red

[4] a: white; b: brown

Table 2. Relative discriminability of three butterfly model-mimic pairs from an avian UVS and VS visual perspective

			Model					
			♂			♀		
	Mimic		a	b	c	a	b	c
UVS	*Prioneris sita*[1]	♂	0.32	-	-	4.74	-	-
			0.60	2.05	2.89	1.63	3.00	4.17
		♀	7.35	-	-	0.11	-	-
			3.30	2.40	1.42	1.14	2.70	2.45
	Hypolimnas misippus[2]	♀	1.92	0.58	-	2.38	0.64	-
			1.02	0.19	-	1.17	1.11	-
	Papilio polytes f. *romulus*[3]	♀	4.50	21.49	-	3.93	6.40	-
			4.36	17.22	-	3.40	12.71	-
VS	*Prioneris sita*[1]	♂	0.52	-	-	1.79	-	-
			0.57	2.11	3.08	1.16	2.28	4.46
		♀	1.92	-	-	0.39	-	-
			0.66	2.67	1.51	0.17	2.70	2.55
	Hypolimnas misippus[2]	♀	1.80	0.75	-	2.32	0.58	-
			0.71	0.25	-	0.93	0.63	-
	Papilio polytes f. *romulus*[3]	♀	4.11	20.94	-	3.63	6.86	-
			3.82	20.48	-	3.11	15.23	-

For each species, the top and bottom rows represent relative discriminability of dorsal and ventral wing colouration, respectively. Letters (a-c) indicate different wing colour patches (see below).

[1] Model: *Delias eucharis*; a: white; b: yellow; c: red

[2] Model: *Danaus chrysippus chrysippus*; a: white; b: orange

[3] Model: *Pachliopta hector*; a: white; b: red

Table 3. Relative discriminability of butterfly models and mimics in *genutia* mimicry ring

Model		*Danaus g. genutia*				*Cethosia mahratta*			
		♂		♀		♂		♀	
Mimic		a	b	a	b	a	b	a	b
Elymnias caudata	♀	0.09	0.83	0.83	1.29	4.16	3.48	1.29	4.12
		0.32	0.88	1.45	1.44	4.04	1.63	1.55	4.48
Arygnnis hybrida	♀	3.09	3.75	5.38	3.13	1.37	2.97	0.74	-0.52
		3.50	3.99	6.69	3.45	2.61	0.79	1.81	0.17

For each mimetic species, the top and bottom rows represent relative discriminability of dorsal wing colouration from an avian UVS and VS visual perspective, respectively. Letters indicate different wing colour patches (a: white; b: orange).

Table 4. Relative discriminability of butterfly models and mimic in *aristolochiae* mimicry ring from an avian UVS and VS visual perspective

Model		*Pachliopta pandiyana*				*Pachliopta aristolochiae*			
		♂		♀		♂		♀	
Mimic		a	b	a	b	a	b	a	b
Papilio polytes romulus f. *stichius* (♀)	UVS	-	-	0.57	3.04	2.57	1.88	4.03	1.69
		-	-	1.98	0.40	2.74	9.89	3.77	7.61
	VS	-	-	-0.07	3.99	2.54	1.44	3.57	1.81
		-	-	1.01	0.40	2.81	11.15	3.53	8.10

For each type of avian vision, the top and bottom rows represent relative discriminability of dorsal and ventral wing colouration, respectively. Letters indicate different wing colour patches (a: white; b: red). Missing values were due to the inability to calculate intraspecific colour distance (which was needed to derive relative discriminability) because of sample size of one specimen.

Table 5. Relative discriminability of butterfly models and mimics in *Euploea* mimicry ring from an avian UVS and VS visual perspective

	Model		*Euploea core core*				*Euploea klugii kollari*				*Euploea sylvester coreta*			
			♂		♀		♂		♀		♂		♀	
	Mimic		a	b	a	b	a	b	a	b	a	b	a	b
UVS	*Hypolimnas bolina jacintha*	♀	7.38	0.44	7.72	0.35	-	-	4.18	0.50	6.16	0.33	5.65	-0.03
			2.92	-0.01	3.50	-0.16	-	-	3.47	0.26	3.83	0.35	4.31	-0.05
	Papilio c. clytia f. *clytia*	♂	4.76	1.99	5.35	1.77	-	-	4.89	1.70	9.22	1.66	7.94	1.02
			0.84	1.38	1.65	0.76	-	-	1.24	1.86	2.19	2.26	1.84	1.11
		♀	2.98	0.78	3.42	0.51	-	-	3.32	1.28	6.75	0.98	5.78	0.32
			0.37	0.40	0.66	0.07	-	-	0.62	0.95	0.86	0.85	0.91	-0.02
	Papilio dravidarum	♂	2.86	0.83	3.33	0.57	-	-	3.18	1.23	6.77	0.95	5.63	0.35
			1.29	-0.15	1.52	-0.49	-	-	1.60	0.12	1.67	0.50	2.11	-0.14
		♀	2.39	0.24	2.55	0.16	-	-	0.84	-0.03	2.61	-0.10	1.75	-0.32
			1.47	0.24	1.70	-0.17	-	-	1.80	0.45	1.83	0.88	2.35	0.33
VS	*Hypolimnas bolina jacintha*	♀	2.85	0.92	2.96	0.93	-	-	1.32	1.17	4.21	0.43	2.63	0.03
			2.41	0.02	2.87	-0.12	-	-	2.23	0.20	3.84	0.57	3.16	-0.37
	Papilio c. clytia f. *clytia*	♂	4.06	3.48	4.32	3.34	-	-	3.36	3.70	8.40	2.44	5.35	1.60
			1.07	2.24	1.53	1.54	-	-	1.32	2.57	2.69	3.51	1.84	1.21
		♀	1.98	1.00	2.13	1.04	-	-	1.67	1.52	4.75	0.63	2.85	0.15
			0.33	0.56	0.39	0.42	-	-	0.31	0.96	0.69	1.23	0.57	-0.07
	Papilio dravidarum	♂	2.79	0.92	3.02	0.93	-	-	2.39	1.05	6.64	0.35	4.02	0.02
			0.90	0.01	1.03	-0.30	-	-	0.50	0.17	1.48	1.00	1.10	-0.22
		♀	1.30	0.82	1.43	0.77	-	-	1.00	0.56	4.19	0.11	2.12	-0.18
			0.82	0.39	0.89	-0.19	-	-	0.38	0.44	1.23	1.34	0.96	0.03

For each mimetic species, the top and bottom rows represent relative discriminability of dorsal and ventral wing colouration, respectively. Letters indicate different wing colour patches (a: white; b: brown). Missing values were due to the inability to calculate intraspecific colour distance (which was needed to derive relative discriminability) because of sample size of one individual.

Table 6. Relative discriminability of butterfly models and mimics in *Tirumala* mimicry ring from an avian UVS and VS visual perspective

	Model		*Tirumala limniace exoticus*				*T. septentrionis dravidarum*				*Parantica aglea aglea*				*Parantica nilgiriensis*			
			♂		♀		♂		♀		♂		♀		♂		♀	
	Mimic		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
UVS	*Papilio c. clytia* f. *dissimilis*	♂	1.99	3.36	-	-	1.45	7.10	1.99	4.97	0.40	8.17	0.44	8.40	1.88	11.72	-	-
			-0.21	5.89	-	-	0.70	5.95	0.66	5.36	0.51	7.80	0.06	9.74	2.97	12.16	-	-
		♀	1.58	2.16	-	-	1.31	5.70	1.55	3.50	0.70	6.93	0.53	7.07	3.10	10.42	-	-
			-0.20	4.28	-	-	0.67	4.00	0.57	3.48	0.42	5.85	0.43	7.46	3.11	10.06	-	-
	Pareronia c. ceylanica	♀	8.32	1.43	-	-	6.00	0.94	7.56	2.55	6.14	-0.44	6.90	0.39	7.92	0.41	-	-
			0.12	0.42	-	-	1.04	-0.10	0.75	-0.34	0.01	1.06	0.89	1.78	3.91	4.77	-	-
	Pareronia hippia	♀	4.88	0.23	-	-	3.27	0.54	4.46	1.48	2.92	-0.20	3.63	-0.26	3.37	1.47	-	-
			0.44	0.03	-	-	1.43	0.30	1.07	0.56	0.13	1.33	1.80	1.75	4.37	3.67	-	-
VS	*Papilio c. clytia* f. *dissimilis*	♂	2.73	4.06	-	-	2.59	8.07	2.61	5.20	1.07	9.03	0.51	9.79	2.11	13.05	-	-
			0.64	6.03	-	-	1.92	5.41	1.94	5.15	0.70	10.86	0.03	10.29	1.11	12.64	-	-
		♀	2.00	2.73	-	-	2.00	6.57	1.98	3.74	0.55	7.55	0.20	8.19	1.35	11.36	-	-
			0.52	4.28	-	-	1.73	3.41	1.75	3.25	0.68	8.21	0.20	7.76	1.15	10.22	-	-
	Pareronia c. ceylanica	♀	2.73	1.64	-	-	2.71	0.45	2.70	1.82	0.83	-0.21	-0.09	0.76	2.44	0.81	-	-
			0.92	0.40	-	-	2.20	-0.36	2.10	-0.34	0.33	2.02	0.92	1.83	2.79	4.53	-	-
	Pareronia hippia	♀	1.51	0.35	-	-	1.74	0.25	1.71	0.74	-0.05	0.00	-0.10	-0.02	2.24	1.66	-	-
			1.35	-0.06	-	-	2.68	-0.33	2.58	-0.34	0.73	2.40	1.82	2.06	3.55	4.25	-	-

For each mimetic species, the top and bottom rows represent relative discriminability of dorsal and ventral wing colouration, respectively. Letters indicate different wing colour patches (a: white; b: brown). Missing values were due to the inability to calculate intraspecific colour distance (which was needed to derive relative discriminability) because of sample size of one individual.