ABSTRACT

Title of Dissertation: USE OF MOLECULAR TECHNIQUES TO ADDRESS THE EVOLUTION OF DISPLAY TRAITS IN THE PTILONORHYNCHIDAE AND OTHER PASSERIFORM SPECIES

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Tests of hypotheses addressing the evolution of complex traits have greatly benefited from advances in the field of molecular genetics. Current molecular techniques allow for the identification of genetic variability, useful in estimating genetic relatedness and potentially explaining phenotypic variation. Here I use molecular data to address the evolution of complex traits within the Ptilonorhynchidae and other Passeriformes. My estimation of a bowerbird molecular phylogeny suggests two highly unlikely scenarios of complex trait evolution (i.e. polygyny, bower construction, decoration use, *etc.*); either polygyny and complex display traits evolved in parallel, or monogamy evolved from non-resource based polygyny, a transition for which no unambiguous examples could be

found, and complex traits evolved once and were lost. Molecular evidence also supports the existence of four *Sericulus* species, dating the radiation to coincide with the upheaval of the central New Guinea mountain range, and suggests plumage coloration may be a labile trait within this group and therefore a poor indicator of species relatedness. Use of ultraviolet (UV) signals in birds is hypothesized to associate with the ability to see UV wavelengths, a trait with a well-documented genetic basis (replacements at key amino acid positions in the short-wavelength-sensitive 1 (SWS1) opsin pigment influence pigment sensitivity and potentially color discrimination). UV signal use may alternatively evolve to match the local light environment. Results from bowerbirds suggest extreme differences in UV reflectance are due to light availability and not differences in UV vision because amino acid sequences in the bowerbirds were nearly identical. Expanding upon this study, I compared SWS1 opsin gene sequences from 134 passeriform species and plumage UV reflectance measurements from 91 of these species. Results from the molecular data are unprecedented; replacements at five amino acid positions are predicted to have occurred nearly simultaneously, suggesting a constraint on UV vision evolution. Additionally, species reflect most intensely in wavelengths to which they are predicted to be sensitive. These results suggest a constraint on UV vision may also constrain the evolution of UV signals in the Passeriformes. These studies highlight the usefulness of molecular data when testing hypothesis of species and trait evolution.

USE OF MOLECULAR TECHNIQUES TO ADDRESS THE EVOLUTION OF DISPLAY TRAITS IN THE PTILONORHYNCHIDAE AND OTHER PASSERIFORM SPECIES

by

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PREFACE

This dissertation contains four chapters followed by appendices and a single reference section. Chapters I, II, and III each contain the following order of manuscript sections: abstract, introduction, methods, results, discussion, and conclusion. Chapter II alters slightly from this format as it has already been published and does not contain a specifically label conclusion section. All table captions are listed together and are immediately followed by their respective tables. Figure and figure captions follow the same format. Chapter IV is presented in an abbreviated format where additional methods, results, and discussion, along with additional tables and figures, are presented in a supplemental material section.

I wish to dedicate this dissertation to my wife Susan
and to both the Zwiers and Howard families
in appreciation of all their love and support.

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TABLE OF CONTENTS

List of Tables viii

List of Figures ix

Chapter I. Detailed phylogenetic analyses reveal unexpected and complex patterns of mating system and display trait evolution in the bowerbirds 1

Abstract 1
Introduction 2
Methods 5
Results 14
Discussion 19
Conclusion 25
Table captions 27
Tables 30
Figure captions 34
Figures 36

Chapter II. Plumage based classification of the Bowerbird genus *Sericulus* evaluated using a multi-gene, multi-genome analysis 39

Abstract 39
Introduction 40
Methods 42
Results 47
Discussion 49
Table caption 54
Table 55
Figure caption 56
Figure 57

Chapter III. Plumage UV reflectance in bowerbird matches light environment while variation in UV opsin is highly constrained 58

Abstract 58
Introduction 59
Methods 64
Results 66
Discussion 68

TABLE OF CONTENTS (cont'd)

Chapter III (cont'd)

Conclusion 73
Table captions 75
Tables 76
Figure captions 78
Figures 79

Chapter IV. A key amino acid change in the Passeriform SWS1 opsin gene accompanies near simultaneous changes at other positions and matches plumage UV reflectance 82

Abstract 82
Introduction 83
Methods 85
Results 86
Discussion 88
Table captions 92
Tables 93
Figure captions 95
Figures 96

Supplemental data 98
Table caption 105
Table 106
Figure captions 107
Figures 108

Appendices

Appendix A- List of taxa included in the bowerbird phylogeny 113
Appendix B- List of primers used to amplify and sequence gene regions for the bowerbird phylogeny 114
Appendix C- Details of polymerase chain reactions used to amplify gene regions for the bowerbird phylogeny 118
Appendix D- Analysis of alternative partitioning schemes using the bowerbird phylogenetic dataset 121
Appendix E- Analysis of alternative bowerbird topologies 123
Appendix F- List of taxa used in the *Sericulus* phylogeny 125

TABLE OF CONTENTS (cont'd)

Appendices (cont'd)

Appendix G- List of primers used to amplify and sequence gene regions for the *Sericulus* phylogeny 126
Appendix H- List of primers used to amplify and sequence the bowerbird short-wavelength-sensitive 1 opsin gene 128
Appendix I- List of passeriform species from which UV reflectance and/or short-wavelength-sensitive 1 opsin gene sequences were collected 129

References 133

LIST OF TABLES

1. Molecular dataset and gene tree summary for the bowerbird phylogeny 30

2. Comparison of trait reconstructions estimated from two alternative bowerbird topologies 31

3. Comparison of trait reconstructions given the new topology 32

4. Summary of mating system transitions and display trait evolution in other bird families with non-resource based polygynous species 33

5. Within *Sericulus* taxa, and among species, divergence estimates 55

6. Description of bowerbird short-wavelength-sensitive 1 opsin gene sequences 76

7. List of taxa and body regions from which UV reflectance measurements were taken 77

8. Variable short-wavelength-sensitive 1 positions and difference in pattern of amino acid replacements in relation to position 90 93

9. Pearson correlation coefficients for short-wavelength-sensitive 1 positions in relation to factors calculated from principle component analyses 94

10. Evidence for UV vision in passeriform species showing sampling bias 106

LIST OF FIGURES

1. Maximum likelihood bowerbird phylogeny 36

2. Maximum likelihood bowerbird phylogeny including museum sampled taxa 37

3. Maximum likelihood phylogeny with mapped mating system and display traits 38

4. Phylogeny, trait evolution, and phylogeography of the genus *Sericulus* 57

5. Short-wavelength-sensitive 1 opsin gene map 79

6. Bowerbird phylogeny with traced UV reflection, amino acid replacements, and habitat 80

7. Reflectance spectra from occluded habitat species 81

8. Family level Barker et al. (2004) phylogeny with mapped short-wavelength-sensitive 1 amino acid residues for positions 81, 84, 85, 90, and 91 96

9. Difference in UV reflection associated with replacements at position 90 97

10. Species level Barker et al. (2004) phylogeny with mapped UV reflectance intensity and short-wavelength-sensitive 1 amino acid residues for positions 78 to 101 108

11. Irestedt & Ohlson (2007) passeriform phylogeny with mapped short-wavelength-sensitive 1 amino acid residues for positions 78 to 101 111

12. Difference in UV reflection associated with replacements at position 86 112

CHAPTER I

Detailed phylogenetic analyses reveal unexpected and complex patterns of mating system and display trait evolution in the bowerbirds

Abstract

Estimates of phylogenetic relationships based on molecular data can reveal important and unexpected patterns in trait evolution. Many bowerbird species (family: Ptilonorhynchidae) are polygynous and have highly complex and unique sets of sexual displays, including bower and decoration use coupled with coordinated dancing and vocal displays. However, three monogamous species within this family, the catbirds, show none of these traits. A previous cytochrome b topology estimates a single evolution of polygyny and complex display, yet relatively weak support at basal nodes and contradictory evidence from non-sexually selected morphological characters, suggests a more detailed re-evaluation of the topology is warranted. Using a larger multi-locus dataset, I find a well supported, but unexpected, basal node resolution in which the polygynous avenue bower-builders are basal to the monogamous catbirds and its sister group, the polygynous maypole bower-builders. This new topology supports the occurrence of one of two scenarios, either of which we show to be extremely unlikely in birds: 1) parallel evolution of polygyny and complex displays in the bower-building species, or 2) a single evolution of polygyny and complex displays in the bowerbird

ancestor with a reversion to biparental monogamy and loss of complex displays in the catbird ancestor.

Introduction

The evolution of complex sexual display traits remains a subject of considerable debate (e.g. Andersson 1994; Borgia 2006; Mead and Arnold 2004). Phylogenetic analyses based on molecular data have helped elucidate the evolution of traits by allowing for an independent assessment of species relationships on which traits can be mapped. Conclusions of trait evolution based on phylogenetic inference are highly dependent on the reliability of the topology to represent true phylogenetic relationships (Baker and Wilkinson 2001; Price et al. 2007). The development of high-throughput molecular techniques with robust phylogenetic methods, allowing for the analysis of multiple loci datasets, have greatly improved estimates of phylogenetic relationships and consequently their value in the analysis of display trait evolution.

The bowerbirds, known for highly elaborate sexual display traits, present a particularly interesting case where accurate phylogenetic analyses are especially valuable when reconstructing the evolution of complex displays. Male bowerbirds have some of the rarest and most complex sexual displays seen in any vertebrate group, in particular, the use of a stick bower unique to bowerbirds (Cooper and Forshaw 1977; Frith and Frith 2004; Johnsgard 1994; Marshall 1954). Stick bowers typically follow one of two forms, either a two-walled avenue design, or a maypole design centered around a sapling (Gilliard 1969). Males of most species build ground courts on which the bower is constructed, and may decorate both the bower and court with objects collected from the

surrounding environment. Males perform coordinated physical displays, with some species highlighting colorful plumage found body-wide or in specific patches located on wings or crests. Physical acrobatics are synchronized with songs comprised of multiple vocal elements. Females search among different male's bowers (Uy et al. 2000; Uy et al. 2001) and males perform their integrated physical displays as females enter or approach the bower. Numerous studies show that these multiple physical and behavioral elements are important in affecting male mating success (e.g. Borgia 1985; Borgia 2008; Coleman et al. 2004; Madden 2002; Patricelli et al. 2002). These highly complex displays are only found in bowerbird species that exhibit a non-resource based (NRB) polygynous mating system, where males contribute only sperm and have no role in parental care. In stark contrast is the relative lack of complex display traits found in the monogamous catbirds (*Ailuroedus*), a three-species genus in the Ptilonorhynchidae in which males contribute to parental care and do not have ground displays. The extreme contrast seen in the use of display traits within the bowerbirds, and the potential to reconstruct a well supported molecular phylogeny afforded by the analysis of a large multi-locus dataset, offer an important opportunity to further study the evolution of complex sexual displays.

Relationships among bowerbird taxa have been hypothesized using morphological (see Frith and Frith 2004) and molecular data (cytochrome b: Kusmierski et al. 1997, see also Borgia et al. 2007 and Endler et al. 2005 that use the same dataset). From the cytochrome b (cytb) data, three bowerbird clades have been estimated, the avenue bower-building clade, the maypole bower-building clade (although not all species in this clade build bowers), and the catbird clade. Kusmierski et al. (1997) estimated a topology in which the catbird clade is basal, after which the two polygynous bower-building clades

diverged (figure 1, inset). This topology suggests the most parsimonious reconstruction of male bowerbird sexual display traits where, assuming a monogamous ancestor and after the divergence of the monogamous catbirds, there was a single evolution of NRB polygyny and the many display traits common in both the avenue and maypole bower-building species. While agreement between this most parsimonious mapping of display traits and Kusmierski et al.'s (1997) cytb topology may suggest support for the basal placement of the catbirds, relatively weak bootstrap support estimated from the cytb sequences calls into question the basal catbird placement. Phylogenies based solely on cytb sequences have been shown to poorly resolve deep nodes (e.g. Moyle 2004), allowing for the possibility that the proposed relationships among the bowerbird clades are incorrect. Additionally, evidence from presumably non-sexually selected characters, such as osteology, number of secondary feathers, and egg coloration, suggests the catbirds are not basal but are instead more recently derived (Bock 1963; see also Frith and Frith 2004). These issues suggest that a re-examination of the phylogenetic relationships among the three major bowerbird clades, and how this affects hypotheses about the evolution of bowerbird mating system and male display, is warranted.

To estimate a new bowerbird phylogeny, I use ten loci, including regions from mitochondrial genes, nuclear introns, and a nuclear exon, and a series of more detailed phylogenetic analyses compared to those used by Kusmierski et al. (1997). Any differences in basal node resolutions, estimated from Kusmierski et al. (1997) and compared to my analyses, would result in a re-evaluation of the evolution of mating system and complex male sexual display traits in the bowerbirds. Specifically, I test whether the pattern of mating system and display trait evolution, given the newly

estimated topology, is equally or less parsimonious than the pattern predicted from the cytb topology. Additionally, if the newly estimated topology results in multiple, equally parsimonious patterns of mating system and display trait evolution, I test for significant differences between these alternative patterns using likelihood analyses. I consider the types of mating system transitions predicted from the alternative patterns, and compare these predictions to what is typically found in other families with NRB polygynous species. Also, I consider the most-parsimonious reconstruction of sexual display traits given each evolutionary pattern, and discuss the role pre-adapted traits (e.g. use of a ground court) may have in allowing rare and complex traits (e.g. use of a bower) to evolve in a more labile, and less parsimonious fashion. Lastly, in addition to re-evaluating previously estimated species' relationships within the bowerbirds, the inclusion of all bowerbird species in the analyses allow me to estimate previously unknown relationships.

Methods

Data collection

Tissue samples that were collected in the field and used by Kusmierski et al. (1993; 1997), were also used in this analysis. Since then, additional field and museum samples have been collected, are included in this paper, and represent all known Ptilonorhynchidae species (see appendix A). Of particular interest is the inclusion of two samples of the recently rediscovered *Amblyornis flavifrons* (see Beehler et al. 2007; Diamond 1982). Additionally, I included three species in this dataset as outgroups based on the Barker et al. (2004) passeriform phylogeny: *Cormobates leucophaeus*, *Malurus*

leucopterus, and *Xanthotis flaviventer*, and differed from that used by Kusmierski et al (1997). I included a total of 52 individuals, representing all known (20) bowerbird species. I performed total genomic DNA extractions on tissue samples following DNeasy tissue extraction protocols (Qiagen). Phenol-chloroform and centrifugal dialysis protocols were used to extract DNA from both toe pad or feather samples from the museum specimens in an isolated and exclusive ancient DNA laboratory (see Fleischer et al. 2000 for a more detailed protocol).

I sequenced a portion of the cytochrome b (cytb) gene for newly sampled taxa, and to resolve ambiguous nucleotide sites from previously reported sequences, I re-sequenced cytb for taxa included by Kusmierski et al. (1997). In addition, I included partial sequences of one additional mtDNA gene (NADH dehydrogenase subunit 2 (ND2)); eight nuclear introns (adenylate kinase (AK) intron 5, β-fibrinogen (Fib) intron 7, glyceraldehyde-3-phosphate dehydrogenase (Gapdh) intron 11, ornithine decarboxylase (ODC) introns 6 and 7, ribosomal protein 40 (RP40) intron 5, transforming growth factor β-2 (TGFβ2) intron 5, tropomyosin α-subunit (Trop) intron 5); and one nuclear exon (recombination activating gene (RAG1) exon 1). These gene regions were selected to resolve both deep and more recently derived nodes to more reliably estimate mating system and display trait changes within the bowerbird radiation.

I used previously designed primers to amplify gene regions from DNA extractions of non-museum sampled individuals (see appendix B for a list of all previously and newly designed primers for all loci). Additionally, to increase the chance of amplifying from museum specimen extractions, I designed internal primers to amplify approximately 200 base pair (bp) fragments. Polymerase chain reaction (PCR) recipes and reaction

profiles were specific for each locus, details of which can be found in appendix C. PCR reactions had final concentrations of 0.2 mM for each dNTP, 0.4 – 0.6 μM for each primer, 1.5 – 2.0 mM $MgCl_2$, 1x buffer, and 1 unit of *Taq*-gold polymerase (Perkin-Elmer). Annealing temperatures varied for each primer pair, but the PCR program generally followed: 97°C hotstart for 7 min, 15 cycles of 94°C for 30 s, 52° – 62°C for 30 s, and 72°C for 1 min, with a final elongation step of 72° for 5 min. I used purified PCR product (Qiagen or ethanol precipitate protocols) in 20 μL cycle sequencing reactions following BigDye Terminator v 3.1 protocols, and an ABI 377 acrylamide gel and ABI 3100 capillary sequencers to obtain sequences. I aligned gene regions using Sequencher v.4.7 (GeneCodes, Ann Arbor), and manually checked for gap placement minimizing nucleotide polymorphisms and gap length. Lengths of individual gene regions are reported in table 1.

To address both the resolution of basal nodes and estimate relationships among all bowerbird species, I constructed two datasets. The first dataset, specifically designed to resolve basal nodes, includes all sequenced gene regions for 16 of the 20 bowerbird species and is 9001 bps in length. The remaining four species (*Sericulus ardens*, *Se. aureus*, *Se. bakeri*, and *Amblyornis flavifrons*) were sampled from museum specimens. To estimate relationships among the museum-sampled and closely related species, I created a second dataset that includes all 20 bowerbird species. Because of a lack of overlapping sequences when including the museum samples, the second dataset totals 2621 bps after missing data were removed. For each dataset, I concatenated gene regions using MacClade v4.08 (Maddison and Maddison 2005).

Phylogenetic Analyses

I estimated models of nucleotide substitution for each gene and genome region, for use in partitioned analyses, and for both the 9001 bp and 2621 bp datasets, and an "all but RAG1" dataset (see table 1) using Modeltest v3.7 (Posada and Crandall 1998). I determined the "best-fit" model using two different criteria. According to the An Information Criterion (AIC; Akaike 1974), the best model is one that minimizes the difference between the true probability distribution and that estimated by the model, while the Bayesian Information Criterion (BIC; Schwarz 1978) suggests the model with the maximum posterior probability is best. Both a version of the AIC method, corrected for small sample sizes and represented as AICc (Burnham and Anderson 2002), and the BIC method requires an estimation of sample size (n). Here I use the length of the dataset to signify n (e.g. to analyze the 9001 bp dataset, $n = 9001$). When models selected by AICc and BIC differed for the same dataset, I used the model with the smallest AICc/BIC score (Huelsenbeck et al. 2004; Yang et al. 2000). For both the 9001 bp and 2621 bp datasets, both the AICc and BIC model selection criteria suggest a general time reversible (GTR) model, incorporating an estimate of invariant sites (+ I) and gamma distribution (+ G), as the "best-fit" model.

To estimate phylogenetic relationships, I used maximum parsimony (MP), minimum evolution (ME), Bayesian probability, and maximum likelihood (ML) analyses. I estimated MP topologies using equally weighted, 100 random start, heuristic tree-bisection-reconnection (TBR) searches in PAUP* v4.0b10 (Swofford 2004). I treated gaps as a fifth nucleotide state, as this treatment has been shown to result in more accurate topologies (Ogden and Rosenberg 2007). I also evaluated distance based trees in

PAUP* v4.0b10 (Swofford 2004), evaluated using ME and implementing the nucleotide substitution models estimated in Modeltest v3.7 (Posada and Crandall 1998). I estimated support for both MP and ME distance trees from 1000 bootstrap replicates (heuristic searches for MP) with a value of 70 or higher representing significant nodal support (Hillis and Bull 1993).

I used MrBayes v3.1.2p (Altekar et al. 2004; Ronquist and Huelsenbeck 2003) to estimate posterior probabilities for the most likely topology. Settings for the Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) runs differed depending on the dataset used. For each analysis, I first tested for appropriate sampling among chains by performing initial 10 000 generation runs with temperatures ranging from 0.003 to 0.2. I selected a temperature in which the proportion of chain swapping among all chains was between 20 to 80 percent (for the non-partitioned 9001 bp dataset, I used a temperature of 0.04). Second, four chains were analyzed in each of two runs (four runs to analyze the non-partitioned 9001 dataset) with data being sampled once every 1000 generations. Third, I used the average standard deviation of the split frequencies to determine when an appropriate number of generations had been run (I ran the analysis for the non-partitioned 9001 bp dataset for 5×10^7 generations). Lastly, while likelihood values reached near stationarity (stabilized around an average of zero) early in the runs (<1 million generations), I used a “burnin” of 50% when summarizing parameter and tree data to ensure early tree searching results did not bias the later analysis. In addition, I used Tracer v1.4 (Rambaut and Drummond 2007) and AWTY (http://ceb.csit.fsu.edu/awty; Wilgenbusch et al. 2004), respectively, to test for adequate parameter and tree sampling. Posterior probabilities of 0.95 or greater were used to indicate significant support.

Because there is a potential that posterior probabilities are artificially inflated (Suzuki et al. 2002), I also used GARLI (Zwickl 2006) to estimate maximum likelihood (ML) topologies and bootstrap support. To estimate the tree with the best GARLI score, I replicated a best tree search 100 times. I estimated support for the GARLI ML tree from 500 (for gene and gene group analyses) or 1000 (for both the 9001 and 2621 bp datasets) non-parametric bootstrap replicates.

Since MrBayes v 3.1.2p (Altekar et al. 2004; Ronquist and Huelsenbeck 2003) allows for assignment of specific evolutionary models to different data partitions, I further tested whether alternative partitioning schemes altered topology estimates. Initial analyses using the 9001 bp dataset were based on individual gene and genome partitions, using options in MrBayes to unlink partitions. Additional partitioning strategies included treating nuclear introns separately or grouped, nuclear exons separately or grouped and treating all codons the same or different, and mitochondrial genes separately or grouped again treating all codons the same or different (see appendix D). To decide between alternative partitionings, Bayes factors were calculated by comparing harmonic mean log likelihood scores estimated in MrBayes (see Brandley et al. 2005; Sullivan and Joyce 2005).

I also tested alternative topologies in relation to the final ML topology. Likelihood scores for alternative topologies constrained only at basal nodes were estimated from the non-partitioned 9001 bp dataset in MrBayes. I used three methods to test for significant differences between likelihood scores: the Shimodaira-Hasegawa test (Goldman et al. 2000; Shimodaira and Hasegawa 1999) using RELL optimization with 1000 bootstrap replicates in PAUP*, the approximately unbiased test in CONSEL v0.1j

(Shimodaira and Hasegawa 2001), and an analysis of Bayes factors. In addition, analyses of insertions and deletions (indels) have been suggested as an independent analysis of the molecular data (Simmons and Ochoterena 2000). I therefore compared the presence of indels among species, and relationships suggested by these data, to the alternative topologies.

Trait mapping analyses

To address the evolution of mating system and complex sexual display traits in the bowerbirds, I mapped presence of NRB polygyny and seven display traits for each bowerbird and outgroup species onto the newly estimated bowerbird topology using Mesquite v2.6 (Maddison and Maddison 2009). The seven display traits, found in both of the two bower-building clades but not commonly found in other avian species, include use of a bower, cleared court, crest presentation display, exploded lek, ground display, separation of sexes, and use of decorations. To allow for the possibility that these display traits do not evolve independently of mating system, I tested for a correlation between mating system and each of the seven display traits using Pagel's (1994) correlation method as implemented in Mesquite v2.6 (Maddison and Maddison 2009; Midford et al. 2002). Significance was determined using an alpha of 0.05. Since these traits are expected to be highly non-independent, I did not use an alpha corrected for multiple comparisons when testing for independence, as an uncorrected alpha would result, in this case, in a more conservative test. I found that all display traits were significantly correlated with NRB polygyny, and were therefore pooled into a single trait called "polygyny and complex display" and coded as either present or absent. This pooled trait was used in all further trait reconstruction analyses.

With these data, I first wanted to test which alternative topology, the topology estimated by Kusmierski et al. (1997) or the newly estimated topology, predicted the more parsimonious and more likely reconstruction of "polygyny and complex display". Both topologies were based on the 9001 bp likelihood analysis. The topology estimated by Kusmierski et al. (1997) was constructed from the newly estimated topology by moving the catbird clade to be basal to the sister bower-building clades. The inclusion of museum-sampled taxa and branch lengths were dealt with in two ways, first by including all taxa including those sampled from museum specimens in the topology and setting all branch lengths equal to one, and alternatively by excluding museum-sampled taxa from the topology and using ML estimated branch lengths. I compared reconstructions of "polygyny and complex display" among the alternative topologies by counting the number of evolutionary steps in the most parsimonious reconstruction. Additionally, I tested if one of the topologies resulted in a significantly more likely reconstruction. To do this, I began by determining which model of trait evolution was best, a symmetrical model where gains and losses are equally likely, or an asymmetrical model where gains and losses are estimated separately. Likelihood scores for these models were compared using a likelihood ratio test, where degrees of freedom equals the difference in the number of parameters (df = 1), and through the calculation of AICc scores, where n equals the number of species (n = 23). Using these analyses I tested whether the newly estimated bowerbird topology resulted in a significantly less probable and less likely reconstruction of mating system and display traits compared to the earlier cytb-only bowerbird topology (Kusmierski et al. 1997).

If multiple reconstructions of "polygyny and complex display" are possible given the newly estimated topology, I also wanted to test if one reconstruction was more parsimonious or more likely than another. I mapped the pooled trait onto the final ML topology estimated from the 9001 bp dataset, retaining the ML-estimated branch lengths. I included the museum-sampled species, following placement estimated from analyses of the 2621 bp dataset, setting branch lengths for these species equal to one. I first wanted to know what reconstruction had the fewest number of steps when assuming either the presence or absence of "polygyny and complex display" in the bowerbird ancestor. Then using a likelihood analysis, I tested whether the ancestral state for "polygyny and complex display" could be statistically resolved. To do this, I again tested for the best evolutionary model using a likelihood ratio test and comparisons of AICc scores. Then using the best model, estimate proportional likelihood values for the presence of "polygyny and complex display" in the bowerbird ancestor. After performing these analyses, if no pattern of trait reconstruction can be determined to be more probable than another, I investigate whether the patterns in mating system transitions and sexual display trait evolution estimated when assuming either a monogamous or polygynous bowerbird ancestor, are commonly found in other avian families with NRB polygynous species. In this way, I am able to thoroughly address the evolution of NRB polygyny and sexually selected display traits in the bowerbirds given the newly estimated topology.

Results

Phylogenetic analyses

While an unweighted MP analysis of cytb does not resolve basal nodes, a ML analysis of cytb agrees with Kusmierski et al.'s (1997) results; that is catbirds (CB) lie basal to the avenue (A) and maypole (M) bower-building sister clades (CB(A,M)), albeit with little bootstrap support (table 1). Analyses of the mtDNA dataset (cytb and ND2 combined; found under combined: mtDNA in table 1) using MP, ME, or ML do not resolve basal relationships with any significant support. While several of the nuclear introns individually resolve the node of interest with varying support, analyses of the combined nuclear intron dataset strongly support a topology where the avenue bower-builders are basal to the catbird and maypole bower-building sister clades (A(CB,M)). Additionally, MP and ML analyses of the combined mitochondrial and nuclear intron only dataset (not including RAG1) strongly support A(CB,M), as do analyses based solely on RAG1. Using the full 9001 bp dataset, both MP with a bootstrap value of 96 and ML with a posterior probability of 1.00 and a bootstrap value of 95 supports the A(CB,M) topology.

To determine whether alternative partitioning schemes estimate different topologies with better likelihood scores, I calculated Bayes factors in reference to the partitioning scheme with the most partitions (P31) to quantify my change in opinion for which scheme(s) estimated the most likely tree (appendix D). In all cases, Bayes factors suggested a change in opinion in favor of the most partitioned dataset. Yet when I compared topologies, I found no differences in the resolution of species' relationships. This suggests that differences in likelihood scores are due to the addition of model

parameters and not because of a better tree estimation. Therefore, all final analyses using either the 9001 or 2621 bp dataset were performed without defining data partitions.

The approximately unbiased (AU) test performed in CONSEL, a Bayes factor analysis using harmonic mean likelihood scores, and an analysis of indels all supported the A(CB,M) topology over CB(A,M). Results from the AU test suggested A(CB,M) is the best topology and that CB(A,M) is worse ($p = 0.049$). A Bayes factor score of 14.52 suggested a change of opinion in favor of A(CB,M) over CB(A,M). Additionally, three indels provided information pertaining to the resolution of the basal node of interest, with all three indels supporting the A(CB,M) topology. No indels provided support for either of the alternative topologies (i.e. CB(A,M) or M(CB,A)). While results from the Shimodaira-Hasegawa (SH) test in PAUP* suggest A(CB,M) is the best topology, it was not significantly different from CB(A,M), ($p = 0.062$). Details of the Bayes factor and indel analyses can be found in appendix E.

Topology summary

When analyzing the 9001 bp dataset, I find that the catbirds are sister to the maypole bower-builders, and that the avenue bower-builders form the basal clade with high bootstrap support in both the MP and ML topologies, and with high posterior probabilities (figure 1). The approximately unbiased test, Bayes factors associated with constrained topologies, and an independent indel analysis all support this topology. Additionally, my results place tooth-billed bowerbirds (*Scenopoeetes dentirostris*) at the base of the maypole bower-builders with strong support. The golden bowerbird (*Prionodura newtoniania*) is the next most recently diverged taxa after the tooth-billed bowerbirds, making the streaked bowerbird (*Amblyornis subalaris*) the basal *Amblyornis*

species. The genus *Amblyornis* is paraphyletic, with *Archboldia papuensis* placed sister to *Am. inornatus* with strong support. The *Sericulus* genus is the basal avenue bower-building genus followed by *Ptilonorhynchus*. The *Chlamydera* species form a monophyletic group where the previously excluded Western bowerbird (*Chlamydera guttata*) is sister to the spotted bowerbird (*Ch. maculata*). Also, the white-eared catbird (*Ailuroedus buccoides*) is the basal catbird species. Analyses of the 2621 bp dataset, which includes the museum-sampled species, place the golden-fronted bowerbird (*Am. flavifrons*) sister to *Am. macgregoriae* (figure 2), albeit with weak support. Lastly, placement of *Sericulus* as the basal avenue bower-building genus is supported from both ML bootstrap values and posterior probabilities, but relationships among the *Sericulus* species are unresolved.

Test of hypotheses concerning trait reconstruction

To test hypotheses of trait reconstruction, I first simplified my analyses by testing for a correlation between presence of polygyny and each of the seven sexual display traits. Using Pagel's (1994) correlation method and an alpha of 0.05, I find that all traits significantly correlated with mating system. I then pooled all traits with mating system into a single trait called "polygyny and complex display", where all polygynous species were coded as "present" and all monogamous species were coded as "absent" (figure 3).

I then tested whether the reconstruction of the pooled trait on the newly estimated topology (A(CB,M)) was significantly worse than the reconstruction estimated from the cytb topology (CB(A,M)) proposed by Kusmierski et al. (1997). Using the CB(A,M) topology and assuming a polygynous bowerbird ancestor results in the most parsimonious reconstruction overall, requiring only a single evolutionary step (table 2).

In comparison, mapping “polygyny and complex display” onto the A(CB,M) topology results in two equally parsimonious scenarios, each requiring two evolutionary steps. In the likelihood analysis, comparison of alternative models using likelihood ratio tests reveal that neither model has a significantly more likely score, yet the lower AICc score for the symmetrical model suggests it is the better-fit model. When comparing results across topologies, regardless of model or how I treated the museum-sampled taxa and branch lengths, the CB(A,M) topology consistently results in the smallest AICc scores (table 2).

From the above analysis, I find that the newly estimated topology results in two equally parsimonious reconstructions (scenarios) describing the evolution of mating system and complex display traits within the bowerbirds (figure 3). Scenario M assumes a monogamous bowerbird ancestor, leading to the parallel evolution of NRB polygyny and complex display traits in each of the bower-building clades. Alternatively, scenario P assumes a NRB polygynous bowerbird ancestor with complex display traits and a reversion to monogamy and loss of complex display traits in the catbirds. In testing whether either scenario is more likely, I estimated proportional likelihood values for presence of the pooled trait in the bowerbird ancestor. Neither model (symmetrical or asymmetrical) had a significantly more likely score (although the symmetrical model had a smaller AICc value; table 3) so I estimated proportional likelihood values using both models. Neither model though, could statistically resolve the ancestral state of “polygyny and complex display”.

Since neither parsimony nor likelihood analyses could significantly support scenario M nor scenario P, I investigated how common the evolutionary changes required

in each scenario are in other avian families with NRB species. Specifically, I looked for evidence of transitions from NRB polygyny to monogamy as suggest by scenario P, and evidence for multiple evolutions of cleared ground courts, decoration use, and bower construction as suggested by scenario M. Payne (1984) lists 16 avian families in which at least one species, males form leks and have been shown, or are expected to exhibit, NRB polygyny. I performed a literature search to find the most recent molecular phylogenies for each family (two phylogenies combined two families listed in Payne 1984, i.e. Cotingidae and Oxyruncidae, and Phasianidae and Tetraonidae, resulting in a total of 14 analyzed families/groups). Onto these phylogenies I mapped the known mating system for each species. Results from these data show no unambiguous examples of NRB polygyny to monogamy transitions (table 4). Additionally in these 14 families/groups, use of a ground court evolved at least once in seven of the groups (50%), decoration use evolved at least once in two of the groups (14%), and bower construction evolved only in the bowerbirds (7%). Thus the probability of evolving all of these traits, as seen in the bowerbirds, is 0.5%. Not only do I find very weak evidence that monogamy has evolved from NRB polygyny, as suggested in scenario P, but also that the probability of evolving a ground court, decoration use, and bower a second time, as suggested in scenario M, is very low.

Discussion

Basal node resolution

Results from my phylogenetic analyses suggest unique relationships among the three bowerbird clades. My results provide strong evidence that the catbirds are sister to

the maypole bower-builders with a basal placement of the avenue bower-builders, with both high bootstrap support and posterior probabilities. This topology is further supported by three separate hypothesis-testing analyses (the approximately unbiased test and analyses of Bayes factors and indels) and evidence from other non-sexually selected traits (Frith and Frith 2004, Bock 1963). While resolution of species within the clades differs only slightly compared to earlier estimates, the well-supported placement of the catbirds sister to the maypole bower-builders does not agree with previously estimated bowerbird molecular topologies (e.g. Kusmierski et al. 1997).

Mating system and display trait evolution

My topology based on molecular data, hypothesizes several unexpected and unlikely patterns in bowerbird mating system transitions and sexually selected display trait evolution, patterns which are very different than what was predicted from earlier bowerbird phylogenetic analyses. Kusmierski et al.'s (1997) cytb topology suggests the most parsimonious evolutionary pattern, requiring only a single evolution of NRB polygyny and complex display traits from a monogamous ancestor (figure 1 inset). Given that the newly estimated topology, based on a multi-locus, multi-genome dataset and using current phylogenetic analyses, is a more accurate representation of the true species tree, my results instead suggest that one of two equally parsimonious, but less parsimonious than that predicted from the cytb-only topology, scenarios occurred in the evolution the bowerbird of mating system and sexual display traits. Not only are these scenarios less parsimonious than suggested from earlier analyses, but they each also require a highly unusual evolutionary event to occur, i.e. parallel evolution of extremely

rare traits (bower) and similar integration of rare traits into complex displays (ground display) in scenario M, or a transition to monogamy from NRB polygyny in scenario P.

The unlikeliness of scenario M results from the hypothesized parallel evolution of NRB polygyny and complex displays in each of the bower-building clades. NRB polygyny has been suggested to have independently evolved multiple times in other avian families (e.g. Bleiweiss 1998), suggesting that parallel evolution in the bowerbirds may not be that unexpected. Yet unlike other avian families, parallel evolution of NRB mating in the bowerbirds is associated not only with parallel evolution of the individual display traits, some of which are found only in the bower-builders (i.e. bower use, vocalizations, physical acrobatics), but also the similar integration of these traits into complex displays (while there is species-specific variation in male ground display, all males typically collect decorations to place on or around their bower, and have a display that includes physical acrobatics coordinated with vocalizations). I show that while some families with NRB species have evolved use of ground courts, very few also use decorations as in the bower-builders, and only the bower-building bowerbirds have evolved the use of a bower. This shows that bowerbirds have indeed evolved a very unique set of highly complex display traits, yet whether these traits can evolve multiple times within the family in unclear.

Evidence from other avian species argues for the labile evolution of sexual display traits (e.g. plumage in orioles, see Omland and Lanyon 2000). This suggests that the sexual displays seen in the bowerbirds may evolve in a labile fashion, allowing for multiple evolutions of these traits within this family. Yet the bowerbird sexual display traits are highly complex. Cunningham et al. (1998) and Omland (1999) suggest that the

probability of evolving complex traits is very low, arguing that parallel evolution of the complex traits seen in the bower-builders is unlikely. Specifically in reference to parallel evolution of the complex and exceedingly rare trait of bower use, certain environmental pre-conditions (i.e. lack of ground predators (see Diamond 1986) and the evolution of traits pre-adapted for bower use (i.e. ground court and decoration use), may have allowed bower use to evolve multiple times within the bowerbirds. The lack of these pre-conditions and pre-adaptations may explain why bower use is nonexistent in other species. Still, if multiple traits serve as pre-adaptations for bower use, parallel evolution of bower use would require parallel evolution of the suite of pre-adapted traits. Therefore, while the requirement in scenario M for parallel evolution of NRB polygyny is not unreasonable, it seems very unlikely that the highly complex sexual display traits associated with NRB polygyny in the bowerbirds evolved in parallel in each of the bower-building clades.

The alternative scenario P also suggests an evolutionary change for which there are no unambiguous examples of other avian families, a reversion from NRB polygyny to monogamy in the catbirds. Approximately 92% of avian species exhibit a socially monogamous mating system (Lack 1968), and while multiple evolutions to polygyny have been documented (Temrin and Sillén-Tullberg 1994), from my literature search I find little evidence for transitions from NRB polygyny back to monogamy in birds. From the 16 (reduced to 14 in my analysis) families listed in Payne (1984), where at least one species shows evidence for NRB polygyny, it is clear that in most of these families NRB polygyny evolved either just once, or multiple times with no clear reversions to monogamy (Indicatoridae, Menuridae, Paradisaeidae, Psittacidae, Pycnonotidae,

Tyrannidae, Cotingidae/Oxyruncidae, Ploceidae, Scolopacidae, and Trochilidae; for references see table 4). Within the Pipridae, classification of mating systems for *Antilophia galeata* and some *Lepidothrix* and *Xenopipo* species is unknown so mating system evolution is unclear. Also unclear are results within the Otididae, as published phylogenetic reconstructions are contradictory. Lastly, NRB polygyny may have evolved deep within the Phasianidae/Tetraonidae phylogeny, but additional data on species-specific mating systems and statistical support at important phylogenetic nodes (see placement of *Lagopus*) are needed to support the evolution of monogamy from NRB mating in this group of species. From these studies, I find no unambiguous examples showing the reversion to monogamy from a NRB polygynous mating system, suggesting a reversion to monogamy in the catbird ancestor as suggested in scenario P would be unique and therefore predicted to be highly unlikely.

Associated with a reversion to monogamy in the catbirds is the loss of complex display traits found in the bower-builders. It has been argued that loss of a trait once it has evolved is more likely than evolving the trait initially, particularly in the case of extremely complex traits (Cunningham et al. 1998; Omland 1999) such as those found in the bower-builders. This would suggest that while the number of evolutionary steps in the evolution of complex displays is the same between scenario M and P, scenario P with one gain and one loss is more probable than the two independent gains hypothesized in scenario M. Yet associated with a reversion to monogamy in the catbirds are likely also changes in both female preferences and male traits (Cunningham and Birkhead 1998). While few details of catbird behavior have been published, male catbirds are territorial (Diamond 1986) and there is some degree of male parental care (Frith and Frith 2004).

Additionally, the lack of complex traits found in the catbirds suggests that female catbird preferences shifted away from traits based solely on genetic benefits to presumably include preferences for traits based on material benefits. Therefore, while scenario P may be more likely because it requires only one gain and one loss (rather than two gains hypothesized by scenario M), scenario P also requires an undocumented transition in avian mating systems from NRB polygyny to monogamy and numerous changes in both female preferences and male traits.

Being based on a well-supported and well-resolved bowerbird topology, there are two likely scenarios for how the highly complex display traits found in the bower-builders evolved. Regardless of whether either scenario M or P are correct, either scenario requires exceptionally rare and unlikely evolutionary events; scenario M requires parallel evolution of extremely rare and complex traits and integration of the traits into similar displays, while P requires a reversion to monogamy from NRB polygyny, a mating system transition for which there are no unambiguous examples of in birds, and substantial changes in both female preferences and male traits.

Other phylogenetic considerations

My results place the tooth-billed bowerbirds (*Scenopoeetes dentirostris*) at the base of the maypole bower-builders with high support, in agreement with Kusmierski et al. (1997). The golden bowerbird (*Prionodura newtoniania*) is estimated as the next most recently derived species, not in agreement with Kusmierski et al. (1997). Thus the two Australian members of the maypole bower-building clade are basal, with the remaining maypole species found in New Guinea. This suggests a possible Australian origin of the maypole bower-building clade and subsequent dispersal to New Guinea.

Additionally, two of four existing museum skins of the golden-fronted bowerbird (*Amblyornis flavifrons*) were sampled and analyzed. Formerly thought to be extinct, the relationship of this species to other *Amblyornis* species has been unresolved. The golden-fronted bowerbird shares plumage characteristics (i.e. presence of an orange crest) with both the streaked (*Am. subalaris*) and Macgregor's bowerbirds (*Am. macgregoriae*), but builds a maypole bower more akin to the Macgregor's (Diamond 1982). Little else was known about this species until its recent rediscovery in the Foya Mountains of New Guinea by Beehler et al. (2007). Their rediscovery and unresolved relationship to the other *Amblyornis* species makes the phylogenetic placement of the golden-fronted bowerbird of particular interest. My results show the golden-fronted bowerbird is placed within the *Amblyornis* genus as predicted, more recently diverged than the streaked bowerbird and closely related to the Macgregor's bowerbird.

The topology also suggests paraphyly of the genus *Amblyornis* in relation to the Archbold's bowerbird (*Archboldia papuensis*). The Archbold's bowerbird exhibits a fern court, an orchid vine curtain instead of a stick bower, and a ground display different from that of the *Amblyornis* species (Borgia 1995). Yet well supported evidence from my new, and Kusmierski et al.'s (1997), topology that the Archbold's bowerbird is positioned clearly within the *Amblyornis* genus, suggests that it should be included in the genus *Amblyornis* and be renamed *Amblyornis papuensis*. For the remaining *Amblyornis* species, populations of Vogelkop bowerbirds (*Am. inornatus*) from the Arfak and Fak Fak mountains remain significantly diverged, as suggested from significant differences in sexual displays (Uy and Borgia 2000) and from earlier genetic analyses (Kusmierski et al. 1997).

Within the avenue bower-builders, resolution of the taxa are consistent with that estiamted by Kusmierski et al. (1997). The *Sericulus* genus is basal, and agrees with recently published and more detailed phylogenetic analyses performed within this genus (Zwiers et al. 2008, Chapter 2). Lastly, placement of *Ch. lauterbachii* basal to *Ch. cerviniventris* and *Ch. nuchalis* is supported by posterior probabilities but lacks support from ML (bootstrap value of 69) and MP (bootstrap value of < 50) analyses. Resolution among these three species is of interest because *Ch. lauterbachii* shares many similarities with *Ch. cerviniventris* (such as the lack of nuchal crests, seen in the other three *Chlamydera* species), and placement of *Ch. lauterbachii* may therefore have important implications in the evolution of display traits within this genus.

Inferences of species' relationships throughout the topology are based on strong support estimated from MP and ML bootstrap values and from posterior probabilities. While ME neighbor-joining analyses result in similar relationships, distance methods have been shown to have a more difficult time estimating relationships among distantly related species when compared to the MP and ML criteria (Saitou and Imanishi 1989), likely explaining the lack of bootstrap support at some nodes.

Conclusion

While mating system and complex sexual display trait reconstructions based on a previous phylogenetic analysis of the bowerbirds suggests the most parsimonious evolutionary pattern (Kusmierski et al. 1997), results presented here suggest a more complicated evolutionary process. I find two exceptionally unlikely scenarios, either scenario M, with NRB polygyny and the highly complex and integrated sexual display

traits found in the two bower-building clades evolving in parallel, or scenario P, where NRB polygyny and complex sexual display traits evolved once in the ancestor, but were lost in the catbirds co-occurring with a transition to monogamy. Either case suggests mating system and rare and complex display traits are not constrained to evolve in a parsimonious manner.

Table captions

Table 1- Dataset summary and gene tree estimation; lengths of regions reported when not including, and including museum taxa (in parentheses); estimated topologies for individual and combined gene regions (not including museum taxa) using unweighted parsimony and both minimum evolution and maximum likelihood based on estimated models of nucleotide evolution; branch support for the topology is estimated from bootstrap replicates (B) and posterior probabilities (PP) if applicable; A- avenue bower-builders, M- maypole bower-builders, CB- catbirds

Table 2- Results testing for significant differences between reconstructions of "polygyny and complex display" based on two alternative topologies, the CB(A,M) topology estimated from cytochrome b data, and the newly estimated A(CB,M) topology; CB- catbird clade, A- avenue bower-building clade, M- maypole bower-building clade; each analysis was done either by including museum taxa and setting all branch lengths (BL) equal to 1, or by excluding museum samples and using maximum likelihood (ML) estimated branch lengths. **For the parsimony analysis**, "score" is the number of evolutionary steps in the most parsimonious reconstruction of "polygyny and complex display", reported as gains:losses and total number of steps, given the topology, treatment of museum taxa, and assuming either a monogamous (M) or polygynous (P) ancestor. **For the likelihood analyses**, "score" is the -log likelihood value given the topology, treatment of museum taxa and branch lengths, and model of reconstruction; two

alternative models were compared, each using variable evolutionary rates but with one treating gains and losses equally (symmetrical) and the other unequally (asymmetrical); likelihood ratio tests (LRT) were used to determine the best fit model, and AICc scores show which topology results in the most likely reconstruction

Table 3- Results for the most parsimonious and most likely mapping of “polygyny and complex display” given the newly estimated topology (A(CB,M)) using likelihood estimated branch lengths and including museum sampled taxa with branch lengths set to one. **For the parsimony analysis**, “score” is the number of evolutionary steps in the most parsimonious reconstruction of “polygyny and complex display”, reported as gains:losses and total number of steps and assuming either a monogamous (M) or polygynous (P) bowerbird ancestor. **For the likelihood analysis**, “score” is the -log likelihood value for each reconstructed trait given the model; two alternative models, each using variable evolutionary rates but with one treating gains and losses equally (symmetrical) and the other unequally (asymmetrical), were compared using AICc scores (model with smallest AICc score is the “best-fit” model, in bold) and likelihood ratio tests (LRT) with associated *p*-values; proportional log likelihood values indicate probability of presence of each trait at node I (above) and node II (below; see figure 3), no values represent a significant resolution of the ancestral state

Table 4- Results from a literature search identifying evidence for reversions to monogamy from NRB polygyny, and for traits associated with bower use (i.e. ground court and decoration use) that may act as pre-adaptations for bower use; references: 1-

Johansson and Ericson 2003, 2- Johnsgard 1994, 3- Yom-Tov and Geffen 2006, 4- Ericson et al. 2002, 5- Barker et al. 2004, 6- Cracraft and Feinstein 2000, 7- Frith and Beehler 1997, 8- Hoglund 1989, 9- Nunn and Cracraft 1996, 10- Pruett-jones and Pruett-jones 1990, 11- del Hoyo et al. 1997, 12- Merton et al. 1984, 13- Wright et al. 2008, 14- Brosset 1982, 15- Greeney et al. 2006, 16- Miller et al. 2008, 17- Pizo and Aleixo 1998, 18- Tello and Bates 2007, 19- Ohlson et al. 2007, 20- Lislevand et al. 2007, 21- Prager et al. 2008, 22- Baker et al. 2007, 23- Myers 1981, 24- Székely and Reynolds 1995, 25- Bleiweiss 1998, 26- Bleiweiss et al. 1997, 27- Marini and Cavalcanti 1992, 28- Prum 1994, 29- Broders et al. 2003, 30- del Hoyo et al. 1996, 31- Pitra et al. 2002, 32- del Hoyo et al. 1994, 33- Dimcheff et al. 2002, 34- Drovetski 2002, 35- Spaulding 2007, 36- Cooper and Forshaw 1977, 37- Frith and Frith 2004, 38- Gilliard 1969, 39- Marshall 1954

Table 1

Dataset			Maximum Parsimony			Minimum Evolution		Maximum Likelihood		
Gene region	Length in bps		topology	B	Model	topology	B	topology	B	PP
Mitochondria										
Cytb	925	(645)	polytomy	<50	TVM+I+G	polytomy	<50	CB(A,M)	60	1.00
ND2	1091	(734)	CB(A,M)	51	GTR+I+G	polytomy	<50	polytomy	<50	<0.50
Nuclear introns										
AK	635	(0)	A(CB,M)	95	HKY+G	CB(A,M)	53	A(CB,M)	54	0.95
Fib	980	(249)	A(CB,M)	89	TVM+G	A(CB,M)	77	A(CB,M)	88	0.99
GAPDH	363	(235)	A(CB,M)	98	TrN+I	M(CB,A)	53	M(CB,A)	62	0.56
ODC	704	(0)	A(CB,M)	67	TVM	A(CB,M)	86	A(CB,M)	86	1.00
RP40	336	(0)	polytomy	<50	K81uf	CB(A,M)	50	CB(A,M)	<50	1.00
TGFB2	604	(145)	A(CB,M)	88	GTR+I	A(CB,M)	76	A(CB,M)	87	1.00
Trop	491	(0)	polytomy	<50	TIM+I	polytomy	<50	polytomy	<50	<0.50
Nuclear exon										
RAG1	2872	(613)	A(CB,M)	74	TrN+G	A(CB,M)	84	A(CB,M)	87	1.00
Combined										
mtDNA	2016	(0)	CB(A,M)	60	GTR+I+G	polytomy	<50	A(CB,M)	58	1.00
nuclear introns	4113	(0)	A(CB,M)	100	GTR+G	A(CB,M)	97	A(CB,M)	99	1.00
mtDNA+introns	6129	(0)	A(CB,M)	95	GTR+I+G	A(CB,M)	60	A(CB,M)	92	1.00
Total evidence	9001	(2621)	A(CB,M)	96	GTR+I+G	A(CB,M)	68	A(CB,M)	95	1.00

Table 2

	With museum taxa, BL = 1				Without museum taxa, BL = ML estimated			
topology	A(CB,M)		CB(A,M)		A(CB,M)		CB(A,M)	
Parsimony analysis								
ancestral state	M	P	M	P	M	P	M	P
score (gains:losses)	2:0	1:1	1:0	2:1	2:0	1:1	1:0	2:1
total number of steps	2	2	1	3	2	2	1	3
Likelihood analysis								
model	score		score		score		score	
symmetrical	7.673		5.343		6.559		5.807	
AICc	**19.946**		**15.286**		**17.718**		**16.214**	
asymmetrical	7.955		5.416		6.671		5.982	
AICc	**18.100**		**13.022**		**15.532**		**14.154**	
LRT	0.564		0.146		0.224		0.350	
p-value	*0.453*		*0.702*		*0.636*		*0.554*	

Table 3

Parsimony analysis		
ancestral state	M	P
score (gains:losses)	2:0	1:1
total # of steps	2	2
Likelihood analysis		
		node I
model	score	node II
symmetrical	6.68	0.271
AICc	**15.55**	0.797
asymmetrical	6.57	0.303
AICc	**17.74**	0.862
LRT	0.23	
p-value	*0.635*	

Table 4

Bird families with lekking species (Payne 1984)	most parsimonious estimate of mating system evolution	evidence for polygyny	ground court	decoration use	bower use	References
Indicatoridae	monophyly of NRB species	X				1,2,3
Menuridae	monophyly of NRB species	X	X			4,2
Paradisaeidae	monophyly of NRB species	X	X	X		5,6,7,8,9,10
Psittacidae	monophyly of NRB species	X				11,2,12,13
Pycnonotidae	monophyly of NRB species	X				14
Tyrannidae	monophyly of NRB species	X				15,2,16,17,18
Cotingidae/Oxyruncidae	multiple evolutions of NRB	X	X			19
Ploceidae	multiple evolutions of NRB	X	X			20,21
Scolopacidae	multiple evolutions of NRB	X				22,2,23,24
Trochilidae	multiple evolutions of NRB	X				25,26,2
Pipridae	unclear: unknown mating system classification	X	X			27,28
Otididae	unclear: contradictory phylogenetic analyses	X				29,30,2,31
Phasianidae	unclear: lack of phylogenetic support	X	X			32,33,34,2,35
Ptilonorhynchidae	reversion to monogamy, parallel evolution of NRB equally likely	X	X	X	X	36,37,38,2,39

Figure captions

Figure 1- Bowerbird maximum likelihood topology with support for branches described from minimum evolution (ME) bootstrap/maximum parsimony (MP) bootstrap/maximum likelihood (ML) bootstrap/posterior probabilities (PP), where “*” describes ≥ 70 or ≥ 0.95 for bootstrap values and posterior probabilities respectively and “-” describes < 70 or < 0.95 for bootstrap values and posterior probabilities respectively; within species bifurcations are significantly supported by all criteria; genus abbreviations: Ai.- *Ailuroedus*, Am.- *Amblyornis*, Ar.- *Archboldia*, Ch.- *Chlamydera*, Pr.- *Prionodura*, Pt.- *Ptilonorhynchus*, Sc.- *Scenopoeetes*, Se.- *Sericulus*; inset, topology estimated by Kusmierski et al. (1997) where bar indicates single evolution of NRB mating and complex display

Figure 2- Bowerbird maximum likelihood topology including all species and based on the 2621 bp dataset; taxa sampled from museums are in bold; * represents bootstrap support above 70, values are given when support is below 70; black branches represent posterior probabilities ≥ 0.95 and grey represents branches with posterior probabilities < 0.95

Figure 3- Simplified bowerbird maximum likelihood phylogeny, including estimated placement of museum samples (grey lines; see figure 2), and showing alternative mating system transitions and trait reconstructions, M- assuming a monogamous ancestor and hypothesizing parallel evolution of NRB polygyny and complex traits (2 gains), P-

assuming a polygynous ancestor and hypothesizing a gain and loss of NRB polygyny and complex traits; table lists reconstructed traits and presence “1” or absence “0” in each species; behaviors described from Frith and Frith (2004), Coates (1990), and Gilliard (1969)

Figure 1

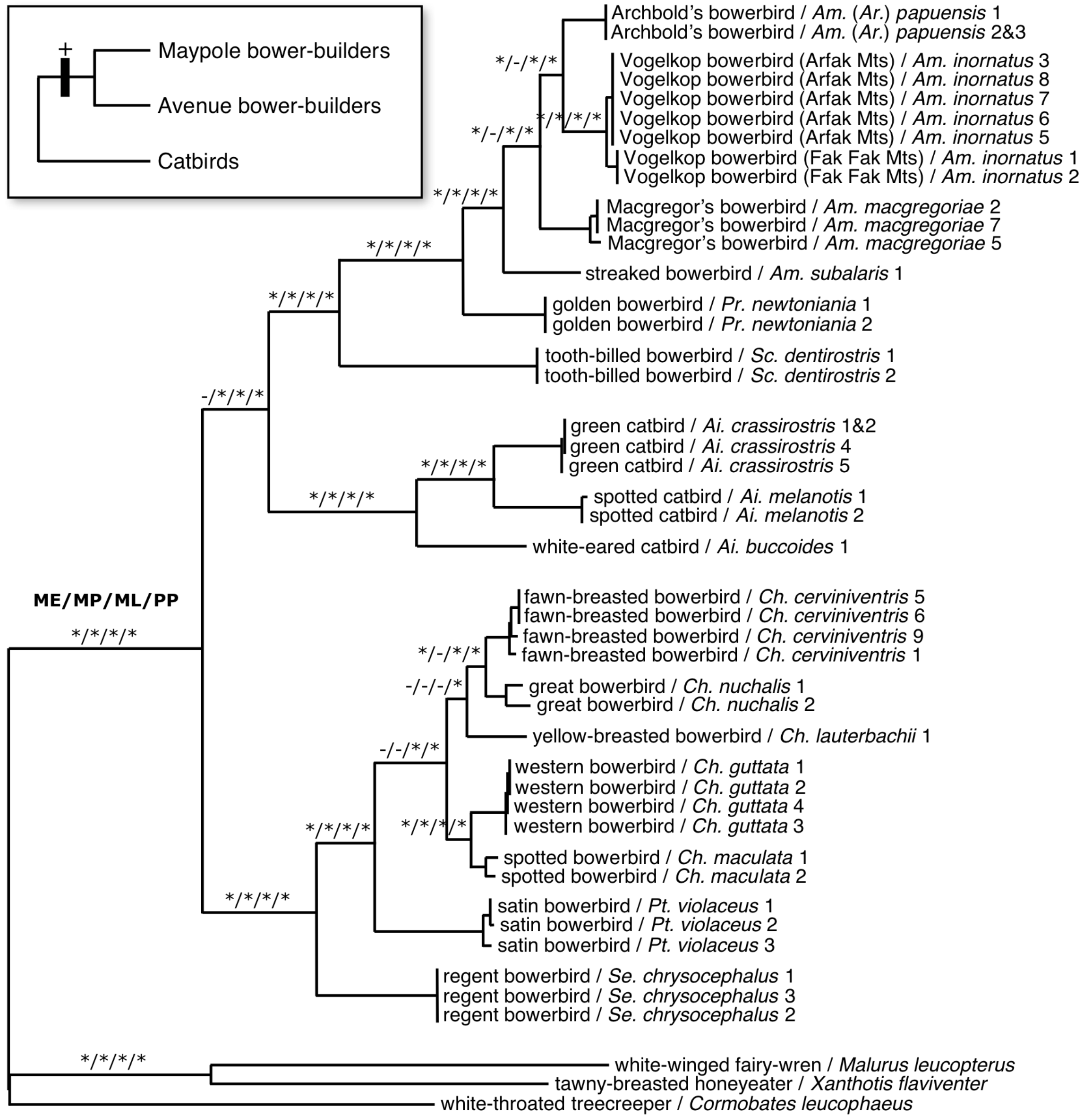


Figure 2

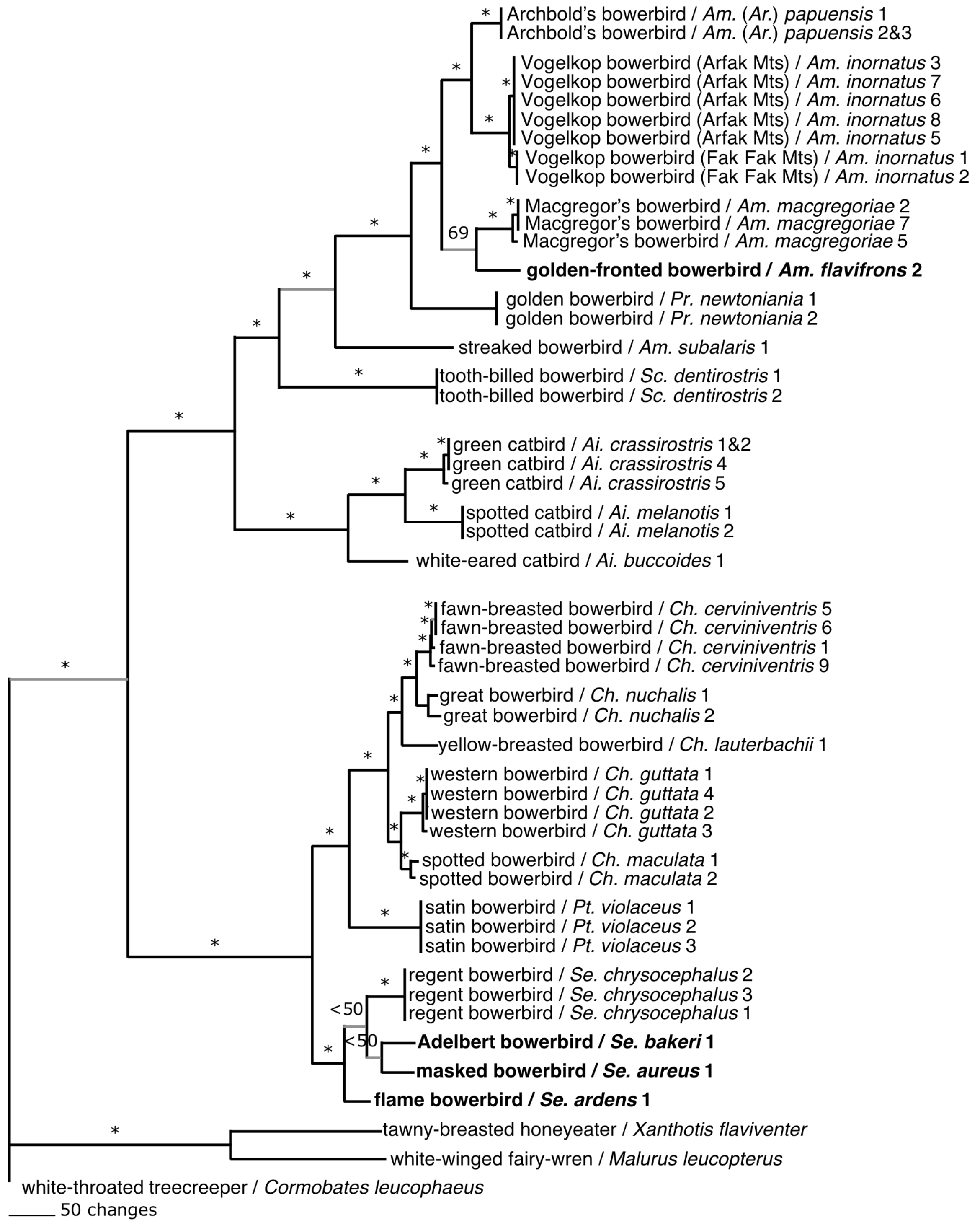
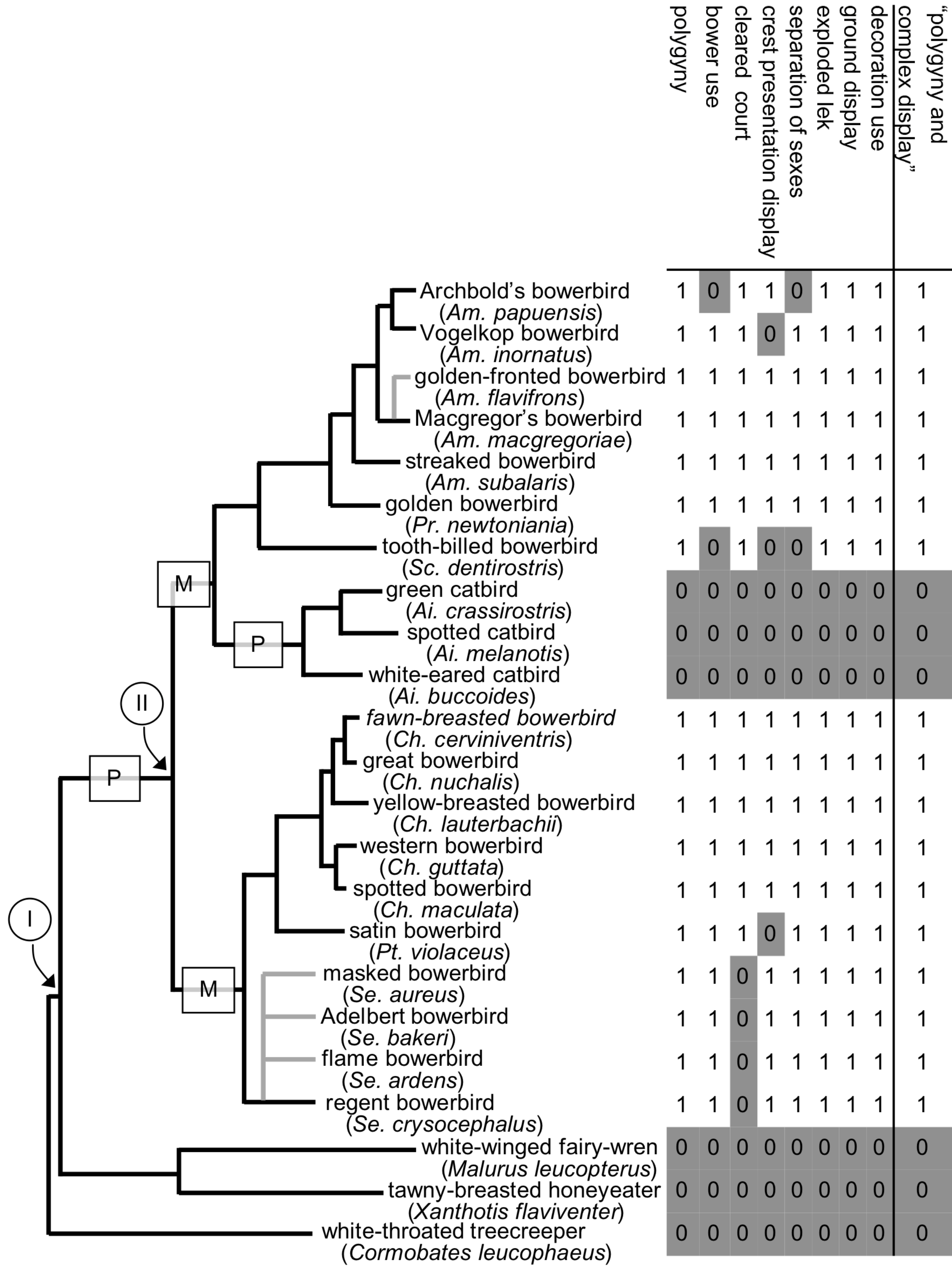


Figure 3



CHAPTER II

Plumage based classification of the Bowerbird genus *Sericulus* evaluated using a multi-gene, multi-genome analysis

Abstract

Past classifications of taxa within the bowerbird genus *Sericulus* (family: Ptilonorhynchidae) conflict since the discovery of hybrids identified though male plumage characteristics. I use molecular data to help define species within this genus and by estimating a phylogeny, test for lability in the evolution of male plumage patterns. Because this genus includes the most brightly colored bowerbird species, and is hypothesized to be the basal genus of the avenue building bowerbird clade, the organization of the four taxa within this genus is especially important in understanding how bowerbird plumage coloration evolved. Analyses of two mitochondrial and six nuclear gene regions confirm the basal placement of *Sericulus* in the avenue building bowerbirds and *Sericulus* monophyly, and suggests the Australian *S. chrysocephalus* is the basal *Sericulus* species. My analysis additionally supports the existence of three New Guinea *Sericulus* species, contrary to some previous plumage based classifications, as they are genetically equidistant from each other. Molecular and geographic data of New Guinea are consistent suggesting a series of speciation events approximately 3.7 – 4.3 million years ago leading to four extant *Sericulus* species. The absence of resolution within the New Guinea species precludes any statements of trait lability, but does suggest

that traits under high selection pressures may not accurately indicate species level distinctions within this genus.

Introduction

The use of phylogenetic analyses based on molecular data has become increasingly important as evolutionary biologists test hypotheses of trait evolution, and in defining species/subspecies limits. While much of the trait evolution research focuses on the effects of different types and degrees of selection intensity, many by mapping trait variation onto a phylogeny, little work has described the effects of selection on traits with large learning versus large genetic components. While other studies have found that traits with a large learning component can be highly labile, here I use molecular data to test for evidence of labile evolution in a sexual selected trait with a large genetic component. In addition, a species concept relying on reproductive isolation can be complicated by the discovery of hybrids. Here, I use molecular data to define species boundaries in a genus where taxa originally ranked as species were considered conspecifics after the discovery of hybrids. In studying the Bowerbird (Ptilonorhynchidae) genus *Sericulus* from Australia and New Guinea, I address both of these issues. By mapping plumage pattern on to my phylogeny, I test whether sexual selection can result in lability in a trait with a large genetic component. In addition, using genetic distances, and a phylogeographic analysis, I address whether classifying *S. ardens* and *S. aureus* as conspecifics due to the discovery of hybrids based on plumage characteristics is appropriate.

Taxa in the genus *Sericulus* exhibit highly elaborate mating displays involving decorated courts and bowers, bright plumage, vocalizations, and physical acrobatics,

traits that are well known among many of the Bowerbird species (Lenz 1994, Lenz 1999, Marshall 1954), and have been shown to be under sexual selection pressures in related species (Borgia 1985, Borgia 1995, Coleman et al. 2004, Patricelli et al. 2002, Uy and Borgia 2000). Males in this genus exhibit the most colorful plumage found in the bowerbirds, expressing vivid yellow and orange in the crown, crest, mantle, and wing in contrast to the black plumage found on the remainder of the body (figure 4; Cooper and Forshaw 1977, Frith and Frith 2004). Evidence from other bowerbird species, and considerable variation among *Sericulus* species in the ratio of colorful to black plumage suggests that the pattern of plumage coloration is highly evolutionarily labile and that classifications based on this trait may be misleading. However, in defining *Sericulus* taxa, male plumage has been routinely used (Bell 1970, Coates 1990, Cooper and Forshaw 1977, Frith and Frith 2004, Ogilvie-Grant 1915).

Originally, four *Sericulus* species were defined, *S. chrysocephalus*, *S. ardens*, *S. aureus*, and *S. bakeri* (see review in Bell, 1970). The discovery of a presumed *S. ardens* and *S. aureus* hybrid (Ogilvie-Grant, 1915) based on intermediate plumage characteristics, in the Wataikwa River Valley of New Guinea, resulted in these taxa being considered subspecies, *S. aureus aureus* and *S. a. ardens*, rather than full species (Beehler et al. 1986, Coates 1990, but see Iredale 1956). Not until Lenz (1999) reported significant differences in four size-related morphological characteristics where these taxa again designated as separate species. Absent from these analyses though is molecular data and any estimates of genetic divergence.

Molecular phylogenetic analyses including the bowerbirds are few. The most recent and complete bowerbird molecular phylogeny includes 14 bowerbird species

(Kusmierski et al. 1997). This topology, including only *S. chrysocephalus* from the genus *Sericulus*, does estimate that this genus is the most basal taxon in the avenue building bowerbirds, an important node when testing the evolution of colorful displays among the bowerbird species. Previous work provides evidence that sexually selected bowerbird traits have a large learned component that may explain their labile evolution (bower design- Kusmierski et al. 1997, and bower decorations- Uy and Borgia 2000). Results from this paper, using a multi-locus, multi-genome, dataset would be the first to show that a sexually selected bowerbird trait with a large genetic component may also evolve in a labile manner. In addition, hybridization between *S. aureus* and *S. ardens* has resulted in these two taxa being considered conspecifics. Here I use divergence estimates, and a phylogeographic analysis, to help define species within this genus.

Methods

Tissue collection and DNA extraction

DNA for this study came from both field and museum samples. Blood samples were collected from three *S. chrysocephalus* and three *Ptilonorhynchus violaceus* adult males from New South Wales, Australia, and two *Scenopoeetes dentirostris* adult males from Queensland, Australia. DNA was extracted using Qiagen extraction protocols. *Ptilonorhynchus violaceus* is the next most closely related species to *Sericulus* and represents where the remaining avenue building species would fall. *Scenopoeetes dentirostris* is the basal species of the maypole builders, a sister clade to the avenue bower building clade, and represents where the remaining maypole building species would fall (Kusmierski et al. 1997). In addition, *Malurus leucopterus* and *Xanthotis*

flaviventer were chosen as outgroups (Barker et al. 2004). Toe pad samples from museum specimens of *S. ardens, S. aureus, S. bakeri*, and *S. chrysocephalus* were collected (Appendix F), and extracted in an ancient DNA Lab using a phenol-chloroform and centrifugal dialysis protocol detailed in Fleischer et al. (2000).

Polymerase Chain Reaction (PCR) and sequencing

Initial amplification and sequencing was done using standard primers for *S. chrysocephalus* and the outgroups for two mitochondrial gene regions, cytochrome b (Cytb) (Kocher et al. 1989) and NADH dehydrogenase subunit 2 (ND2) (Sorenson et al. 1999), and five nuclear regions, β-fibrinogen intron 7 (Fib), glyceraldehyde-3-phosphate dehydrogenase exon 11 and intron 11 (Gapdh), ornithine decarboxylase intron 7 (ODC), ribosomal protein 40 exons 5 and 6 and intron 5 (RP40), and transforming growth factor β-2 intron 5 (TGFβ2) (Primmer et al. 2002) and recombination-activating gene exon 1 (RAG-1) (Barker et al. 2002, Groth and Barrowclough 1999). Additional primers were designed from sequences obtained from *S. chrysocephalus* in an attempt to sequence smaller, overlapping fragments from the museum specimen extractions (Appendix G). PCR reactions and conditions for museum samples followed protocols for amplifying 'ancient' DNA (Fleischer et al. 2000) in 25 μL reactions. For field samples, a similar protocol was used excluding BSA and using annealing temperatures of 55 °C - 58 °C. Amplification was confirmed using gel electrophoreses, after which samples were purified by centrifugation following Qiagen manufacturer protocols. Cycle sequencing of both strands occurred in 20 μL reactions using BigDye Terminator v 3.1 according to ABI protocols, and reactions were analyzed using an ABI 3100 Automated Sequencer. Sequencher (GeneCodes, Ann Arbor) was used to automatically align sequences, after

which they were corrected by sight to ensure proper base calling and gap alignment. A list of samples, museum accession numbers, locales and dates of collection, as well as regions sequenced and associated GenBank (NCBI) accession numbers are provided in Appendix F.

Difficulty in amplifying all regions from ancient samples resulted in regions of missing data for one or more of the museum specimens of each species. While indels were included in the alignments, regions containing ambiguous or missing data, found particularly at the edges of the data sets, were removed before concatenation. Individual ambiguous sites found within the dataset were also removed. In total, 349 base pairs (bps) of Cytb, 533 bps of ND2, 405 bps of Fib, 267 bps of Gapdh, 169 bps of ODC, 215 bps of LRPP40, 143 bps of TGFB2, and 393 bps of RAG-1 remained in the final dataset, making for 882 bps of mitochondrial DNA and 1592 bps of nuclear DNA, totaling 2474 bps. Of these sites, 327 are parsimony informative. To confirm DNA sequence stereotypy among the New Guinea taxa and identify additional variation in *S. chrysocephalus*, five additional museum samples were extracted using similar procedures, PCR amplified, and sequenced for Cytb, ND2, Gapdh, and RAG-1 (Appendix F).

Phylogenetic analyses

Thorough phylogenetic analyses were performed on the original dataset, not including the additional museums specimens used to verify the original sequences due to difficulty in sequencing nuclear data from museum samples. Non-significant partition homogeneity tests when treating all gene regions as separate partitions (P=1.00), treating genomes separately (P=0.91), Cytb and ND2 only and separately (P=0.46), and the nuclear loci only and separately (P=0.15), supported concatenating the individual gene

data sets into a total evidence data set. Afterwards, the Bayesian Inference Criterion (BIC), as implemented in Modeltest (v3.7; Posada and Crandall 1998), was used to estimate nucleotide substitution models for use in the distance, likelihood, and Bayesian analyses. Models were estimated for a non-partitioned total evidence data set (TrN+I+G), a genome partitioned total evidence data set (HKY+I+G for mitochondrial data, HKY for nuclear intron data), and for individual gene regions (HKY+I for CytB; HKY+G for ND2; HKY for Fib, GAPDH, ODC, and LRRP40; K80 for TGFβ2 and RAG-1). Corrected genetic distances were estimated from the non-partitioned total evidence topology using PAUP* (v4.0b10 (Altivec); Swofford 2001), as were relationships estimated using the likelihood and parsimony criteria. The neighbor joining distance tree was estimated with support from 1000 bootstrap replicates, the maximum parsimony topology was estimated through an exhaustive search with support from 1000 branch and bound bootstrap replicates, and the maximum likelihood topology was estimated through a heuristic (tree bisection and reconnection with 10 random starts) search with support from 100 heuristic bootstrap replicates.

Posterior probabilities for branches were estimated using a parallel version of MrBayes (v3.1.2p; Altekar et al. 2004, Ronquist and Huelsenbeck 2003). An analysis was run on each of three data sets, a non-partitioned total evidence data set, a genome partitioned total evidence data set, and a gene partitioned total evidence data set, to identify possible differences in topology resulting from alternative partitionings of the data. The analyses utilized 4 chains set over 2 simultaneous runs and, using Pooch (v1.7; Dauger Research, Inc.) were run across a cluster of four Dual G5 Macintosh computers. The analyses ran for 6, 8 and 15 million generations for the non-partitioned, genome

partitioned, and gene partitioned datasets respectively, to ensure proper exploration of the parameters. A “temperature” of 0.05 ensured adequate chain swapping and a 10% “burnin” was used for each analysis.

The retained data were used to estimate parameters, their variance, and posterior probabilities of nodes within the topology for each analysis. Upon termination, convergence diagnostics were used to determine whether topologies agreed among each run and whether each run had come close to stationarity. The average standard deviation of the split frequencies was <0.001 for each analysis. The standard deviation for each partition that constructs the backbone of the phylogeny, when compared between the two runs, in each of the three analyses, was the optimal value of 0.00. Only standard deviations of within clade bipartitions deviated from zero, being <0.003 in the New Guinea *Sericulus* clade. The potential scale reduction factor (PSRF) for all branches, on average, varied less than 0.001 from the optimal value of 1.00, and PSRF values for parameters used to determine stationarity were all 1.00, in each dataset. While the reliability of PSRF values is contingent on sample size, support for these bipartitions results from each bipartition being observed in both runs.

Tree dating

We utilized a calibration combining two genes, Cytb and ND2, to date nodes within the *Sericulus* phylogeny, necessary for the phylogeographic analyses. To estimate within taxa divergence estimates, I used a dataset containing all samples, including those used to verify original museum samples. Since the expected topology could not be estimated from either 195 bps of Cytb or 346 bps of ND2 alone, I concatenated these data into a single 541 bps dataset from which the expected topology was estimated. Non-

significant differences between topologies with and without an enforced molecular clock, as determined using the likelihood ratio test (p=0.22), suggests these data evolve in a clock-like fashion. Homologous regions of known Kauai creeper (*Oreomystis bairdii*) and Maui creeper (*Paroreomyza mana*) Cytb and ND2 sequences (Fleischer et al. 1998, R. Fleischer unpublished data) were concatenated, a model of sequence evolution (HKY+I+G) was estimated (BIC in Modeltest v3.7; Posada and Crandall 1998), and pairwise distances were estimated. I used the estimated Oahu subaerial (3.5 MYA) and shield building (3.0 MYA) dates (Price and Clague 2002) to calculate mutation rates of 3.1%/million years and 3.6%/million years (see Fleischer et al. 1998 for the rationale and caveats of the approach). These calibration rates were then applied to divergence values corrected by removal of intraspecific variation to account for lineage sorting (Edwards and Beerli 2000) to roughly estimate dates at nodes within the *Sericulus* phylogeny.

Results

Phylogenetic analyses

To verify museum sequences, I compared each sample to at least one additional sample from each species at two mitochondrial and two nuclear loci. For each *Sericulus* species, intraspecific variation was <1%. Topologies estimated under all phylogenetic criteria, rooting with *Malurus leucopterus* and *Xanthotis flaviventer* and using *Scenopoeetes dentirostris* to represent the maypole builders and *Ptilonorhynchus violaceus* to represent the avenue builders, place the non-*Sericulus* ingroup taxa as expected (see Kusmierski et al. 1997) with bootstrap support and posterior probabilities. This supports a monophyletic *Sericulus* clade with *S. chrysocephalus* as the basal

Sericulus species. In addition, it is possible that some gene regions contribute little phylogenetic signal while others contribute substantially more, resulting in a gene or genome biased topology. Yet, by comparing a non-partitioned analysis to both the gene and genome partitioned analyses using appropriate models of sequence evolution, I found no difference in topology and no support for gene or genome bias (likelihood ratio test, $p = 1.00$).

The most likely topology places *S. aureus* sister to *S. bakeri*, as do all Bayesian and distance analyses (figure 4). While there was no significant difference in likelihood values when comparing constrained topologies of *S. aureus* and *S. ardens* to *S. ardens* and *S. bakeri* as sister taxa (likelihood ratio test, $p = 0.92$), finding *S. ardens* sister to *S. bakeri* was 3.5 times more likely when considering posterior probabilities (average among the three Bayesian analyses). Posterior probabilities support the divergence of the New Guinea taxa, but bootstrap and posterior probability values can only support a polytomy among the New Guinea *Sericulus* species. The most parsimonious analysis places *S. ardens* sister to *S. aureus*, but again without statistical support. In addition, topologies estimated from independent mitochondrial and nuclear analyses agree, suggesting that even with large divergence estimates, the mitochondrial data was able to infer appropriate relationships.

Dating of nodes

Divergence values used to estimate dates were estimated from concatenated Cytb and ND2 mitochondrial sequences and included all museum samples. Interspecific divergence estimates among the New Guinea species were similar, ranging from 7% - 10%. Using rates of 3.1 and 3.6% per million years estimated from homologous

Drepanidine sequence, I estimate that the New Guinea *Sericulus* ancestor split from *S. chrysocephalus* 3.7 – 4.3 MYA, and the New Guinea *Sericulus* taxa diverged from each other between 2.5 and 2.9 MYA (table 5). From these data, it is clear that the *Sericulus* species diverged before the Pleistocene.

Discussion

Phylogeny and species limits

Species and subspecies limits in the *Sericulus* genus have been defined on the basis of male plumage color patterns and the discovery of hybrids. I used a dataset of nearly 2500 base pairs of mitochondrial and nuclear DNA sequence data from eight loci to estimate divergences among the *Sericulus* species. This allowed me to define species limits, estimate the first phylogeny to include all *Sericulus* taxa, and to test the lability of plumage pattern evolution. While the most likely topology suggests *S. aureus* and *S. bakeri* are sister taxa, my dataset cannot statistically support this resolution. Divergence estimates among the three New Guinea species are relatively similar, and since there are species level distinctions between *S. bakeri* to each *S. aureus* and *S. ardens*, the similar level of divergence supports ranking *S. aureus* and *S. ardens* each as separate species (see also Fleischer et al. 2006). These data also suggest these species may have evolved from a common ancestor nearly simultaneously, but an analysis containing additional sequence data would be needed to confirm this.

Evolution of color patterns in male plumage

The bowerbirds are an important group with which to test hypotheses addressing evolution of male sexual display traits. Kusmierski et al. (1997) show that while the two

types of bower, avenue and maypole, are found exclusively in one of two bower building clades, variation within these clades show little phylogenetic pattern. This is true even among recently diverged sister species, suggesting this complex trait is highly labile. Bowers, though, are likely have a large learning component, as juvenile males observe adult males at their bowers (G. Borgia personal communication), possibly explaining the labile evolution of this trait. The pattern of plumage coloration on the other hand, is likely to be highly genetically determined, and possibly less likely to evolve in a labile manner. Contradictory evidence though suggests plumage coloration is suggested to evolve rapidly (Haavie et al. 2000, Lande 1981, Omland and Lanyon 2000). I therefore tested whether general patterns of plumage coloration, i.e. black versus yellow and orange, in the *Sericulus* genus are labile by mapping this trait onto the newly estimated phylogeny, predicting a lack of phylogenetic pattern if patterns of plumage coloration are labile. Also, it is important to note that I distinguish between rapid and labile evolution with rapid referring to the rate at which a trait evolves along a lineage, and labile referring to the amount of change among lineages.

The lack of resolution within the New Guinea *Sericulus* species precludes me from directly stating that male plumage patterns evolved in a labile manner. If the ancestral state for the *Sericulus* genus was a higher degree of black plumage, then two separate evolutions of a higher degree of colorful patterns would have occurred leading to each *S. ardens* and *S. aureus*. Alternatively, if the ancestral *Sericulus* state was a lower degree of black plumage, then two separate evolutions of a lower degree of colorful patterns would have occurred leading to each *S. chrysocephalus* and *S. bakeri*. In addition, *S. ardens* and *S. aureus* share a similar color pattern to each other where much

of the body is brightly colored, leaving only the tail and the outer primaries, and the *S. aureus* face and throat, black, yet I show no support for or against referring to these species as sister taxa (figure 4). If further analyses show *S. bakeri* is sister with either *S. ardens* or *S. aureus*, this would suggest that *Sericulus* male plumage patterns do evolve in a labile manner. While my results cannot support or refute labile evolution in this trait, my results do suggest that the use of male plumage coloration, a trait that is likely under high sexual selection pressures, in defining species within this genus seems impractical.

Phylogeography

The New Guinea orogeny, which has been suggested to have started as early as the mid Miocene (Cloos et al. 2005) or as late as the early Pliocene (Dow and Sukamto 1984, Pigram and Davies 1987, Pigram and Symonds 1991) has been suggested to have greatly influenced the biodiversity of New Guinea (Dumbacher and Fleischer 2001, Heads 2001a, Heads 2002, Joseph et al. 2001), and may have played a part in the New Guinea *Sericulus* radiation. *S. chrysocephalus* is found along the Eastern coast of Australia (0-900 meters), *S. ardens* in the Fly watershed of southern New Guinea (0-900 meters), *S. aureus* in Northern and Western New Guinea (900-1500 meters), and *S. bakeri* isolated in the Adelbert Mountains (900-1200 meters) in Northeastern New Guinea (Cooper and Forshaw 1977, Gilliard 1969, Lenz 1999) (figure 4). This radiation of the New Guinea *Sericulus* species may have resulted from isolation caused by the uplift of the central New Guinea mountain range. A phylogeographic analysis showing consistency between dates for the isolation of northern and southern New Guinea populations and inferred dates on my phylogeny would provide evidence that the central New Guinea orogeny resulted in the extant New Guinea *Sericulus* species.

Results of my phylogeographic analysis suggests a likely sequence of events leading to the extant *Sericulus* taxa. As *S. chrysocephalus* is located in Eastern Australia and is the basal *Sericulus* species, and the fact that the vast majority of the remaining non-*Sericulus* avenue builders and outgroups are found in Australia (Gilliard 1969, Simpson and Day 2004), it is reasonable to assume an Australian *Sericulus* ancestor. This suggests, most parsimoniously, that ancestors of the modern New Guinea *Sericulus* species split from *S. chrysocephalus,* approximately 3.7 – 4.3 MYA, and dispersed northward into southern New Guinea. Dates for the orogeny of the central New Guinea Mountains differ, beginning as early as 12 MYA (Cloos et al. 2005) up until five MYA (Dow and Sukamto 1984, Pigram and Davies 1987, Pigram and Symonds 1991), with mountains becoming a barrier to gene flow approximately 3.5 – 4.5 MYA (Heads 2001b, 2002). This range of dates coincides with the dates on the topology for the divergence of the New Guinea species, suggesting the New Guinea orogeny is likely to have played an important role in the radiation of the New Guinea *Sericulus* species. These results support a vicariance model for the New Guinea *Sericulus* species radiation. Based on the unresolved polytomy of the three New Guinea *Sericulus* taxa and nearly simultaneous separation, the vicariance model suggests that the New Guinea *Sericulus* ancestor, once in southern New Guinea, spread across New Guinea before the mountains becoming a barrier to gene flow. Then the uplift occurred, isolating one population in Southern New Guinea, and two populations along the northern coast.

In addition to the phylogeographic analysis are observations of the altitudinal limits of the four *Sericulus* species. Altitudinal limits have been suggested to play an important role in speciation events in New Guinea, as it is likely that shifts to higher

ranges occurred with contemporaneous geological uplift leading to population isolation (Heads 2001a, 2002). *S. chrysocephalus* and *S. ardens* both reside in lowland areas with an altitudinal limit of up to 900 meters. In comparison, *S. bakeri* resides between 900 and 1200 meters and *S. aureus* resides between 900 and 1500 meters. This evidence, and predictions of Heads (2001a, 2002), suggests *S. aureus* and *S. ardens* are not subspecies because they do not share similar ranges in altitude.

Sericulus *classification*

A study of *Sericulus* wing length, tail length, and two measures of bill size support the existence of four *Sericulus* species, i.e. that *S. ardens* and *S. aureus* are separate species (Lenz 1999), but these data do not describe species divergences. Results from my molecular and phylogeographic analyses support the classification of four distinct *Sericulus* species. My data suggests *S. chrysocephalus* is the basal *Sericulus* species, that the three New Guinea *Sericulus* taxa are similarly diverged from each other, suggesting that *S. ardens* and *S. aureus* are separate species. While my data do not suggest one way or another that the evolution of plumage color patterns in the *Sericulus* species is labile, they do suggest that decisions to rank taxa as conspecifics due to hybrids described from highly sexually selected traits should be re-examined using alternative types of characters.

Table caption

Table 5- Divergence estimates within and among species based on 195 bps of Cytb and 346 bps of ND2

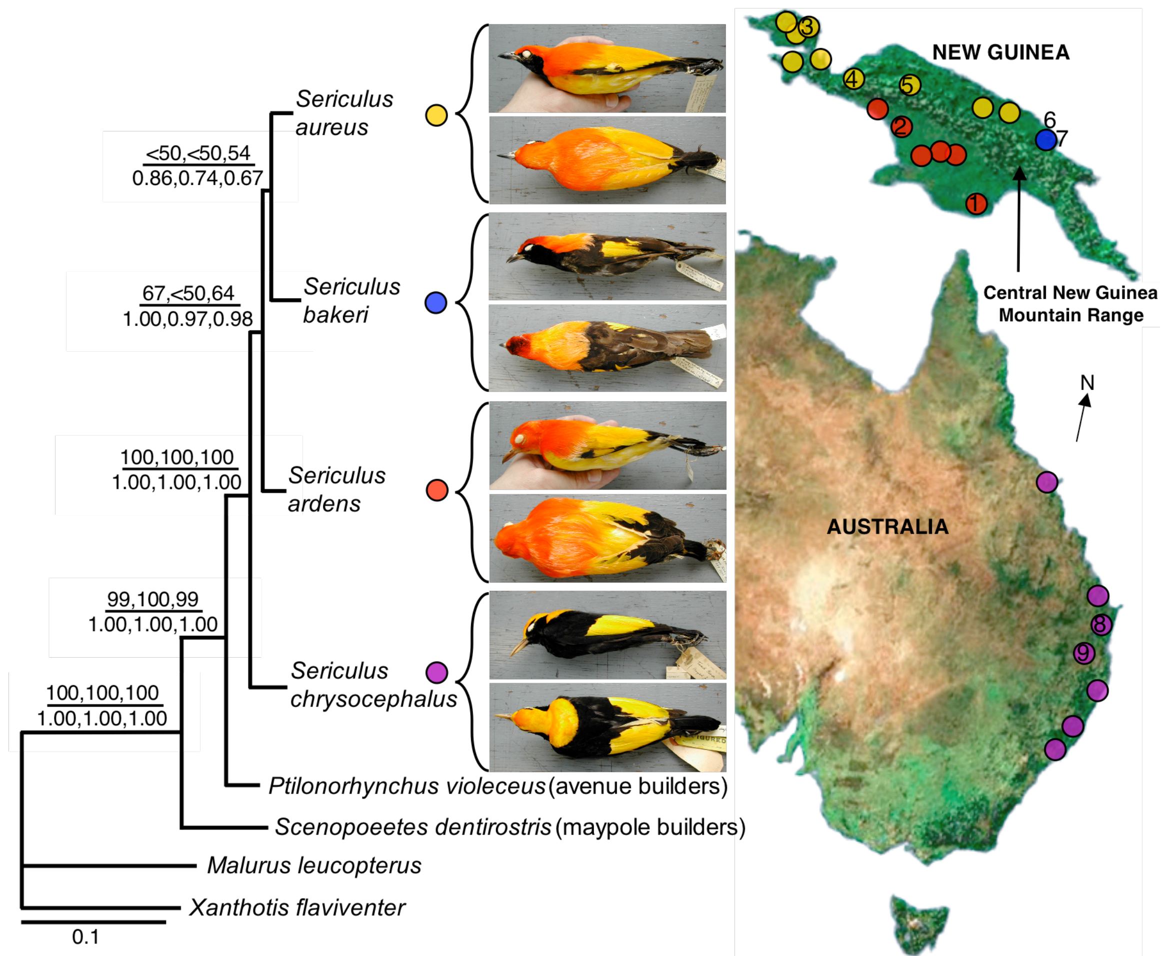
Table 5

	Within species divergence		
Sericulus ardens	0.002		
Sericulus aureus	0.001		
Sericulus bakeri	0.000		
Sericulus chrysocephalus	0.001		
Ptilonorhynchus violaceus	0.007		
	Among species divergence	**3.6% per million years**	**3.1% per million years**
S. aureus, *S. ardens*	0.071	1.89	2.19
S. ardens, *S. bakeri*	0.103	2.81	3.26
S. aureus, *S. bakeri*	0.108	2.97	3.45
NG polytomy	0.094	2.53	2.94
S. chrysocephalus, *S. ardens*	0.143	3.89	4.52
S. chrysocephalus, *S. aureus*	0.134	3.67	4.26
S. chrysocephalus, *S. bakeri*	0.135	3.72	4.32
S. chrysocephalus, NG polytomy	0.137	3.69	4.29
Ptilonorhynchus, *Sericulus*	0.201	5.28	6.13

Figure caption

Figure 4- Maximum likelihood topology based on 2474 bps of mitochondrial and nuclear data; at each node, above line- heuristic maximum likelihood bootstrap (100 replicates), exhaustive maximum parsimony bootstrap (1000 replicates), neighbor joining bootstrap (1000 replicates), below line- posterior probabilities: non-partitioned dataset (6 million generations), genome partitioned dataset (8 million generations), gene partitioned dataset (15 million generations); markers on map identify museum specimen location reviewed by Bell (1970) and Lenz (1999), and are used to represent the range of species; numbers on map coincide with samples used in this paper and referred to in Appendix F

Figure 4



CHAPTER III

Plumage UV reflectance in bowerbirds matches light environment while variation in UV opsin is highly constrained

Abstract

Different hypotheses associated with sensory drive have been proposed to explain variation in plumage characteristics. One hypothesis proposes that differences in the visual system leads to corresponding color-specific male traits. Another hypothesis suggests light availability where males display causes males to evolve plumage that efficiently reflects available light. Recent work suggests the short-wavelength-sensitive 1 (SWS1) opsin gene is responsible for ultraviolet (UV) sensitivity. Sequencing the SWS1 gene for 15 bowerbird species, I found variation at only three species-specific positions that were not within the retinal-binding pocket. The remaining amino acid sequence was identical across all species, including two putatively key positions (86 and 90), residues at which predict a reduced sensitivity to UV wavelengths. This suggests SWS1 sequence variation does not affect UV vision nor would drive differences in male display traits. Reflectance from male bowerbird plumage show that three species in the genus *Chlamydera* have very high UV reflectance from their nuchal crest, whereas other species show much lower UV plumage reflectance. The three highly UV reflecting *Chlamydera* species display in open habitats relative to other species, suggesting a possible important role for UV illumination affecting the use of UV in display. This is the

first study to specifically address SWS1 sequence variation within a single avian family, and particularly one which has significant differences in UV reflection, the results of which suggest male bowerbird UV signal evolution is affected more by ambient UV levels at display sites than by differences in SWS1 sequence.

Introduction

Numerous studies have suggested the importance of ultraviolet (UV) signals in mate choice by birds (Bennet et al. 1996, Andersson & Amundsen 1997, Hunt et al. 1999, Pearn et al. 2001, Siitari et al. 2002), but other studies testing for context-specific UV signals such as mate choice, show that UV reflectance from plumage or other display traits is not used (Borgia 2008, JF Savard pers. communication, Ballentine & Hill 2003, Hunt et al. 2001, Siitari et al. 2007). Yet it remains unclear why some birds use UV signals in mate choice while others do not. From the model of signal evolution proposed by Endler & Basolo (1998), two important and testable hypotheses are derived that may explain differences in vision-based sexual signaling systems. The first hypothesis proposes that male trait coloration is driven by the preexisting visual biases of females. The second hypothesis argues that the colors of male sexual traits are driven by ecological conditions, where the light environment where males display influences male conspicuousness. By comparing male UV reflectance to characteristics of the UV visual system and habitat type across multiple species, I test the importance of these hypotheses in explaining the evolution of UV signals within the bowerbirds (Ptilonorhynchidae).

Sensory drive predicts that differences in visual tuning among species can lead to species-specific differences in colorful display traits. Since UV sensitivity was

discovered in birds (Huth & Burkhardt 1972; Wright 1972), considerable work has explored factors that may influence avian UV vision. The majority of research has focused on the short-wavelength-sensitive 1 (SWS1) opsin pigment, where amino acid replacements at key sites, including positions 86 and 90 (using bovine rhodopsin position numbers; Hargrave & McDowell 1992; Palczewski et al. 2000) are responsible for large shifts in pigment sensitivity of 30 nm or more. The effects of replacements at position 90 are well documented; cysteine (Cys90) results in a UV-biased pigment while serine (Ser90) results in a violet-biased pigment (Yokoyama et al. 2000, Wilkie et al. 2000, Shi et al. 2003, Carvalho et al. 2007). Similar replacements at position 86 may have similar effects on pigment sensitivity, i.e. Cys86 results in UV-shifted sensitivity and Ser90 results in violet shifted sensitivity (Shi et al. 2003), but this result was not confirmed by a later study (Carvalho et al. 2007). It is important to note that avian violet-biased pigments so far discovered have a spectral sensitivity that extends into the UV range, though not as far as UV-biased pigments (Hart & Hunt 2007). Therefore, if male UV reflecting traits evolve to match UV visual abilities within species, I expect different replacements at positions 86, 90, or other sites that might affect the visual system, in species with high plumage UV reflectance when compared to species with low plumage UV reflectance. Hausmann et al. (2003) argue that UV plumage reflectance is associated with tuning in the UV visual system. I would therefore expect to find notable variation in the bowerbird SWS1 if I find differences in plumage UV reflectance. Yet while high variability has been found in plumage UV reflectance from numerous bird species (Eaton and Lanyon 2003), other studies point to the conservative nature of the SWS1 gene as seen in passerine species (Chapter 4). Since no detailed analysis documenting SWS1 variation

within an avian family has been published, it is unclear how variable the SWS1 gene is within an avian family.

The second hypothesis predicts that males evolve colorful traits that are effective signals within the light environment where they display. Previous work has shown the availability of UV wavelengths positively associates with UV reflectance (Gomez & Théry 2004, Endler & Théry 1996), while others have shown the importance of light availability when visualizing colors (Hailman 1977, Lythgoe 1979, Endler 1992, Endler 1993). Uy & Stein (2007) suggest introgression of yellow plumage from the golden-collared manakin into white-collared manakins is due to the greater conspicuousness of yellow than white plumage in light environments through their hybrid zone. Vertical gradients in ambient light are associated with vertical placement of lekking displays in other manakin species (Heindl & Winkler 2003), suggesting the light environment influences male signaling.

Significant differences in levels of available UV wavelengths have been reported among and within habitats (Vézina & Boulter 1966, Smith 1979, Smith & Morgan 1981, Endler 1993) and may explain differences in the level of UV plumage reflectance. Difference in light availability is directly related to the abundance of UV absorbing vegetation (Smith 1979, Endler 1993), resulting in extremely low light levels on forest floors (e.g. in a Queensland, Australia rainforest, only 0.44% of the wavelengths between 400 and 700 nm reach the forest floor, Björkman & Ludlow 1972). Furthermore, UV wavelengths have been shown to be even less common than wavelengths between 400 and 700 nm (standardized irradiance on forest floor at 550 nm, ~ 0.02; at 400 nm, ~ 0.0075; at 300 nm, ~ 0.00125 from Uy & Stein 2007; see also Henderson 1977, Endler

1993). In open habitats, the amount of UV light is more than an order of magnitude greater when compared to woodland habitats (Vézina & Boulter 1966). If open habitats allow for a greater abundance of UV wavelengths and therefore greater illumination of male UV reflecting traits, it is possible that males of species with display sites in open habitats will reflect significantly more intensely in the UV range (between 300 and 400 nm) than males of species found in more heavily vegetated habitats. Alternatively, it is possible that males reflect higher in the UV in more occluded habitats to compensate for the lack of available UV light (see Leal and Fleischman 2002; Fuller and Travis 2004).

I chose bowerbirds as a group to test these hypotheses because species in this family show highly variable and elaborate male trait coloration, seen in both plumage and the use of decorations collected from the environment (Marshall 1954, Gilliard 1969, Cooper and Foreshaw 1977, Frith and Frith 2004). Additionally, males of open-habitat species build display sites in areas with relatively little vegetative cover when compared to the display sites of males of species found in tropical forests, whose display sites are in more densely vegetated areas. Because bowerbird display is concentrated on courts at fixed locations on the ground, the potential role of illumination in affecting display can be assessed more readily than if display occurred at different levels of the forest canopy or if birds followed light patches in the forest.

Studies of the use of UV in sexual displays in bowerbirds have been limited to the Satin bowerbird (*Ptilonorhynchus violaceus*). While initial studies suggested a possible role for UV in plumage (Doucet & Montgomerie 2003) and decorations (Wojcieszek et al. 2006, Mullen 2007), more detailed studies directly assessed how UV reflectance affected mating success and show a more limited role for UV (i.e. blue but not UV from

plumage is used in mate choice, JF Savard pers. communication, and UV reflecting bower decorations are not preferred over otherwise identical UV blocked decorations, Borgia 2008). Satin bowerbird courts are found in forested areas where UV may often be occluded by vegetation, potentially explaining their failure to use UV in sexual display (Borgia 2008). Because bowerbird species build bowers in a wide range of habitats that likely differ in the amount of UV that illuminates the display court, I can compare UV reflectance from male plumage to both SWS1 pigment sequences and the availability of UV inferred from vegetation characteristics where bower display occurs. I exclude bower decorations in this study because they are not designed as signals for bowerbird display, and thus the coincidental UV reflectance of some decoration types may be tolerated if the same decoration reflects a highly valued color that may be the cause of its display on the bower (see Borgia 2008).

The evolution of UV signal use in birds is of considerable interest, but a lack of information describing both UV reflectance and UV vision across multiple species has left key hypotheses addressing the evolution of UV signals untested. Sensory drive proposes male colorful traits may be influenced by both evolutionary and ecological effects. By comparing male UV reflectance to both UV vision inferred from SWS1 sequences and habitat type across the bowerbird family, I test whether the UV visual system or the effects of light availability influence male UV reflectance where males display.

Methods

Measuring UV reflectance

I measured plumage UV reflectance from multiple individuals within 18 bowerbird species, including the two subspecies of *Ptilonorhynchus violaceus*, in rooms with reduced ambient light (table 6). I used an Ocean Optics S2000 spectrometer using a PX-2 xenon light source and a bifurcated cable combining both source lighting and sensor into one probe. A black metal block held the probe at a 45-degree angle to the feather surface allowing for measurements of diffused wavelengths and further reducing ambient light contamination. PTFE tape was used as a white reference (Peddle et al. 2001), and a dark reference was made by covering the sensor and unplugging the light source. For each skin, an initial survey of the plumage was made to identify UV reflecting patches. Here I define UV plumage patches as areas of continuous UV reflecting plumage defined by body regions, i.e. the crown and crest of *Se. aureus* form a continuously colored patch but were measured separately. I excluded patches with exposed underplumage, as well as feathers on the belly and the undertail-coverts as they are likely not used in signaling but are typically light in color and may reflect in the UV (Burkhardt 1989; Eaton & Lanyon 2003). I averaged spectra from three measurements taken from multiple locations within each patch. To identify true reflectance peaks, reflectance values at each .35 nm were smoothed over 10 nm in each direction, with the closest wavelengths contributing proportionally more to the overall value. Spectra from identical patches across individuals within a species were then averaged. I report the maximum value of UV intensity (as a percentage relative to the white standard) across patch types for each species. In doing so, the number of patches measured within a

species does not influence among species comparisons as maximum intensity, and not average intensity, across all patches is used to describe a species' overall UV reflectance. I present maximum UV reflectance measurements in 10 nm intervals from 300 to 400 nm (see Siitari et al. 2007).

DNA extraction and SWS1 sequencing

DNA extractions were performed following Qiagen DNA extraction protocols on tissue samples from 15 of the 20 bowerbird species (Chapter 1) for which tissue samples were available. Using the zebra finch SWS1 sequence (GenBank accession no. AF222331) sequenced from cDNA, I designed primers to sequence the bowerbird SWS1 gene in four segments with large overlapping regions (figure 5, appendix H). Particular attention was paid to the second transmembrane region (TMR), where positions 86 and 90 are found (Palczweski et al. 2000). I used 25 μL PCR reactions with final concentrations of 0.2 mM for each dNTP, 0.4 μM for each primer, 1.5 mM $MgCl_2$, 1x *Taq* buffer, and 1 unit of *Taq*-gold polymerase (Perkin-Elmer). Annealing temperatures varied slightly for each primer pair, but the PCR program generally followed: 97°C "hotstart" for 7 min, 15 cycles of 94°C for 30 s, 62°C for 30 s decreasing .4°C per cycle, and 72°C for 1 min 15 s, 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min 15 s, with a final elongation step of 72° for 10 min. I sequenced the amplified products using 1/8th BigDye v 3.1 terminator cycle sequencing reactions following ABI protocols and an ABI 3100 automated sequencer. I aligned the sequences to the Zebra finch reference sequence, using Sequencher (v 4.7 GeneCodes, Ann Arbor), and to the SWS1 protein alignment suggested by Stenkamp et al. (2002), from which the introns and exons, including TMRs and loops, were inferred.

Results

Plumage reflectance

I find extreme differences in the intensity of UV reflectance from male plumage among the included bowerbird species (figure 6), where three *Chlamydera* species reflect highly in the UV, but the remaining species reflect below 20% in reference to the white standard. Since two UV visual systems are predicted from the effect replacements at position 90 have on UV pigment spectral sensitivity (Yokoyama et al. 2000, Wilkie et al. 2000, Shi et al. 2003, Carvalho et al. 2007), I report both range and mean for two approximations of the avian visual system, a UV-biased (300-400 nm) and violet-biased (350-400 nm) visual system. Intensity of UV reflectance from the nuchal crests of *Chlamydera guttata*, *C. maculata,* and *C. nuchalis*, (50.63% to 137.17% [range, UV-biased], 85.13% [mean, UV-biased]; 68.14% to 80.42% [range, violet-biased], 75.26% [mean, violet-biased]) exceeds the intensity of UV reflectance measured from the plumage patches from the remaining species, see figure 6 (1.79% to 19.99%, 7.88%; 1.79% to 18.90%; 7.80%). The two remaining *Chlamydera* species, *C. cerviniventris* and *C. lauterbachii*, do not have a nuchal crest and reflect with less intensity than the other three crested *Chlamydera* species and more similar to the other remaining species (5.92% to 18.90%, 13.21%; 12.63% to 18.90%, 16.10%). Comparing averages of maximum UV reflectance, I found that the three crested *Chlamydera* species reflected significantly more intensely than the remaining bowerbird species at each 10 nm wavelength interval (two sample t-Test assuming unequal variances, all p-values <0.0001 and significant after bonferroni correction).

SWS1 sequence

I sequenced a total of 2315 aligned base pairs (bps) of the SWS1 opsin gene from each of 15 bowerbird species. Primer set A sequenced TMR II that includes positions 86 and 90, key sites shown to have among the greatest affects on SWS1 protein spectral sensitivity. For all bowerbird species studied, a cysteine was found at position 86 (Cys86) and a serine was found at position 90 (Ser90). SWS1 amino acid sequences were identical across all species except for three positions with unique, species-specific replacements. A Val to Ile replacement at position 104 (Val104Ile) within *Amblyornis papuensis* is the only replacement within a bower-building species, and falls outside TRMs within extracellular loop 1. The other two replacements, Val63Ile in *Ailuroedus crassirostris* and Leu173Phe in *Ai. buccoides*, both occur in catbirds and at positions where it is unclear if they are within TMRs (Stenkamp et al. 2002; Carleton et al. 2005). Regardless, these changes are not likely within the retinal binding pocket (Carleton et al. 2005) and therefore do not likely affect pigment sensitivity.

From the aligned SWS1 sequences, distinct patterns of variation can be identified within the different gene regions (table 7). Introns are highly variable, as expected, in both nucleotide substitutions and presence of insertions/deletions. TMRs on the other hand are highly conserved, even at 3rd codon synonymous positions. Additionally, primer pair C resulted in multiple amplification products, a problem that numerous redesigned primer pairs did not resolve. Individual bands were extracted and purified using Qiagen protocols, and while several unique sequences were obtained, none matched previously sequenced SWS1 gene regions through BLAST searches. The inability to sequence across this region resulted in the amplification and sequencing of 27 bps from the 5' end

of TMR V, and 63 bps from the 3’ end of intracellular loop 3 but not between these points.

Mapping of traits

To address whether replacements at positions 86 or 90, or light environment defined by habitat varies with UV reflection, I mapped these traits onto a newly estimated bowerbird topology (Chapter 1). While I find no amino acid replacements at positions 86 or 90, I do find that the three intensely UV reflecting *Chlamydera* species do not form an exclusive clade (figure 6). Bowerbirds reside in a variety of habitats, with most species found in heavily vegetated habitats except for several *Chlamydera* species that are generally found in more open habitats (Simpson & Day 2004, Cooper and Foreshaw 1977, Frith and Frith 2004). When habitat type is mapped onto the current bowerbird phylogeny (Chapter 1), I find UV wavelengths are intensely reflected only in species that are found predominantly in open habitats but not in other species found in more occluded habitats (figure 6).

Discussion

Extreme differences in male UV reflectance suggest the possibility of a differential use of UV courtship signals among bowerbird species. I tested two hypotheses associated with sensory drive to explain variation found in male UV reflectance. The first posits that male UV reflectance will correlate with replacements at key sites within the SWS1 protein sequence that enable greater UV sensitivity. Hausmann et al. (2003) assume an association between UV vision and UV reflectance, so it is expected that variation in UV reflectance should match variation in the visual

system, here described from SWS1 opsin gene sequences. Across the species that were sequenced, only three amino acid positions show species-specific replacements (positions 63, 104, and 173). They are found outside or at the edge of the TMRs, are not included in the retinal-binding pocket (Stenkamp et al. 2002; Carleton et al. 2005) and at positions that have not been shown to influence UV vision (i.e. positions 46, 49, 52, 86, 90, 93, 114, 116, and 118 in Yokoyama et al. 2000, Wilkie et al. 2000, Yokoyama & Shi 2000, Shi et al. 2001, Fasick et al. 2002, Cowing et al. 2002, Carvalho et al. 2007). Additionally, the three species that have unique replacements reflect little UV and reside in heavy forested habitats, and any effect these replacements may have on UV vision therefore cannot explain the extreme differences I see in UV reflectance. I was unable to sequence across the third exon, and am therefore missing some of TMR V, intracellular loop 3 and the regions in between. Yet, due to their distance from the Schiff base (modeled from Palczewski et al. 2000), replacements within these regions are not expected to influence pigment sensitivity.

The bowerbird radiation is approximately 25 million years old, estimated from corrected cytochrome b sequence divergences (Chapter 1) using a 2% per million year mutation rate (Weir and Schluter 2008, Fleischer et al. 1998) suggesting a high degree of conservation within the SWS1 gene. A lack of replacements at key SWS1 positions suggests that all bowerbirds have identically sensitive UV pigments and possibly identical UV visual abilities. This is consistent with previous work showing that the terrestrial visual system is highly conserved even among related species found in different habitats (Leal and Fleischman 2002, references therein). Therefore, replacements within the SWS1 amino acid sequence that effect UV pigment sensitivity

do not relate to the extreme differences I find in UV reflectance from plumage among the bowerbird species, as suggested by the first hypothesis.

From the positions known to greatly influence SWS1 pigment sensitivity, results from position 90 suggest all bowerbird species have pigments that are violet-biased, i.e. are less sensitive to short UV wavelengths. While all SWS1 pigments in the bowerbirds are also Cys86, which suggests UV-biased pigment sensitivity, the effect of Cys86 is uncertain. *In vitro* experiments suggest Cys86 results in UV-biased sensitivity (Shi et al. 2003) while recent results analyzing pigments from wild-caught birds suggest Cys86 results in violet-biased sensitivity (Carvalho et al. 2007). These results suggest bowerbirds are less sensitive to shorter UV wavelengths, but direct measures of pigment absorption are required to resolve this.

Multiple factors have been shown to influence UV vision aside from SWS1 sensitivity (Hart et al. 2000a, Hart et al. 2000b, Hart 2001; Ebrey & Koutalos 2001, Hart & Vorobyev 2005; Kelber et al. 2003; Cuthill et al. 2000), including transmission properties of the ocular media (cornea, lens, aqueous and vitreous humors), density of cones and UV pigments on the retina, and neural interpretation of visual signals. This allows for the possibility that species-specific variation in these factors, possibly associating with light availability, explains differences in UV vision and results in a preexisting bias for UV reflecting displays. Some studies have begun modeling species-specific UV visual abilities by combining the effects of multiple factors (Hart and Vorobyev 2005, Vorobyev et al. 1998, Hästad and Ödeen 2008, for review see Osorio & Vorobyev 2005), but little is known about how ocular media or the other factors affect vision in the bowerbirds. Therefore, while I find no differences in replacements at key

sites suggesting identical UV pigment sensitivity across the bowerbirds, other factors such as ocular media transmission and cone density may explain the extreme difference I find in UV reflectance, specifically by blocking UV vision in species that also do not reflect highly in the UV.

The second hypothesis states male signals evolve to match light conditions where they display. Bowerbird display sites are found in a variety of habitats, suggesting differences in the availability of UV wavelengths that could be reflected from male plumage. I find that extreme differences in UV reflectance do associate with habitat, where intense UV reflectance is found in species that display in open habitats but not from species in more closed habitats with presumably less UV illumination. This suggests the evolution of male UV reflecting traits is influenced by the light environment where they display, in contrast to other studies showing UV reflectance is more intense in forested species (Leal and Fleischman 2002). Within each habitat, localized light environments are expected. For other bird species found in forests, light spots may provide adequate illumination of male traits with only limited UV attenuation (e.g. Gomez & Théry 2004, Uy & Stein 2007), but because bowerbirds are constrained to display in the immediate vicinity of their bower (Borgia 1986, Borgia 1995, Coleman et al. 2004, Patricelli et al. 2002, JF Savard pers. communication) they are less able to track light spots, which would result in inconsistent and unreliable illumination of UV dependent signals (see Borgia 2008). In open habitats, there may be more direct, and because of less dense vegetation, more diffused UV illumination allowing for adequate illumination of UV reflecting patches.

Differences in UV reflectance among bowerbird species are better explained by differences in light environment than differences in UV vision defined by SWS1 replacements. In the twelve bowerbird species found in more heavily vegetated habitats, I found low intensity of UV plumage reflectance relative to the three highly UV reflecting *Chlamydera* species that live in more open habitats. Violet biased SWS1 pigments from other bird species are partially sensitive to UV wavelengths (Yokoyama et al. 2000) so it appears that the bowerbird SWS1 pigments can detect UV signals in habitats when UV light is abundant. Recent studies of mate choice and plumage reflectance show a positive relationship for blue even though reflectance was low, i.e. 8-12% in satin bowerbirds (JF Savard pers. communication) and ~6% in black grouse (Siitari et al. 2007). The importance of low reflectance values in these studies suggests the possibility that bowerbird species with UV reflectance could still use UV for signaling. However, research has shown that wavelengths from 300 to 400 nm, more so than wavelengths from 400 to 700 nm, are lacking on the forest floor (Henderson 1977, Endler 1993, Uy & Stein 2007), suggesting that UV reflecting patches are less well illuminated and more difficult to see than blue reflecting patches.

Since the level of UV wavelengths on forest floors is very low, what explains the low, but still measurable levels of UV reflectance found from the occluded habitat species? Several papers have noted that carotenoid based colors, mainly associated with yellow and red, also show a characteristic peak in the UV range of around 20% reflectance (Bleiweiss 2005, Hofmann et al. 2007). Of the occluded habitat species, the majority of reflectance is between 500 and 700 nm corresponding with yellow to red coloration. In addition these patches show a low level of reflectance in the UV range

(figure 7). This suggests that UV reflectance from these occluded habitat species may be a side effect of selection for carotenoid-based colors, and not UV signals.

Mapping intensity of UV reflectance, SWS1 replacements, and habitat type onto a recently estimated bowerbird phylogeny reveals the three highly UV reflecting species are recently derived and found within a monophyletic *Chlamydera* clade (figure 6). Within this clade, *C. cerviniventris* and *C. lauterbachii* are found in habitats somewhat similar to the other highly UV reflecting species (*C. cerviniventris* is found primarily in riparian woodland but also at times more open habitats, G. Borgia pers. observation), but do not have nuchal crests. This suggests a most-parsimonious reconstruction of UV signal evolution requiring two gains, one in the *C. maculata* and *C. guttata* ancestor and another leading to *C. nuchalis*. Interestingly, during sexual displays males of all *Chlamydera* species tilt their head towards the female exposing their nuchal crest region (Gilliard 1959, Frith & Frith 2004), even *C. cerviniventris* and *C. lauterbachii* (G. Borgia pers. observation) though they do not have nuchal crests. If nuchal crest and head tilt display evolved at the same time, this would instead suggest UV signal use evolved once in the Chlamydera ancestor, but was lost separately in both *C. cerviniventris* and *C. lauterbachii*. Whether this loss of UV signal use occurred with a transition to a more occluded habitat is possible but unknown.

Conclusion

Two alternative hypotheses associated with sensory drive predict that male UV reflecting traits evolve to be conspicuous within the species' UV visual system or within the light environment where males display. In the first study specifically addressing

SWS1 variability within an avian family, particularly once in which extreme differences in plumage UV reflectance are found, SWS1 sequences from 15 bowerbird species suggest identical sensitivity to UV wavelengths. Thus, differences in UV reflectance have not evolved to match my estimate of the bowerbird UV visual system (i.e. replacements at key SWS1 positions). My results do suggest though, that use of UV signals is restricted to areas where adequate UV wavelengths are available to reliably illuminate male UV reflecting patches during sexual display. This suggests that for bowerbirds, the immediate ecological effects of the light environment are more influential to the use of UV signals than the long-term evolutionary effects of the UV visual system.

Table captions

Table 6- Museum specimens and plumage patches from which UV reflectance was measured, not including patches with exposed underplumage, feathers on the belly or the undertail-coverts; N- National Museum of Natural History, A- American Museum of Natural History

Table 7- Amplified SWS1 gene regions based on Stenkamp et al. (2002) blue opsin sequence and Palczewski et al. (2000) rhodopsin sequence; H- helix, I- intracellular loop, E- extracellular loop, dS- synonymous substitution, dN- non-synonymous substitution; calculations do not include ambiguous sites or gaps

Table 6

Species Subspecies	Museum ID	Common name	# of individuals	breast	crest/nape	crown	ear	forehead	mantle	rump	tail	throat	wing
Ailuroedus buccoides	A679748, A679749, A809352, A421010	white-eared catbird	4		x		x		x			x	x
Ai. crassirostris	N278103	green catbird	1		x				x				
Am. inornatus	N98138, N148112, A679509, A679513, A303093, A793056	Vogelkop bowerbird	6	x					x				
Am. macgregoriae	N584871, N584872	Macgregor's bowerbird	2	x	x				x				
Am. papuensis	A705704, A705705, A705707, A705711	Archbold's bowerbird	3			x			x				x
Am. subalaris	N176989, N161751, A330487, A679575, A330486	streaked bowerbird	5	x	x				x				
Chlamydera cerviniventris	A294626, A679180, A330498, A421008, A679164, A679167	fawn-breasted bowerbird	6	x	x							x	x
Ch. guttata	A810989, A679153	western bowerbird	2		x								
Ch. lauterbachii	A816489, A787614, A787617	yellow-breasted bowerbird	3	x	x							x	
Ch. maculata	A679125, A679126, A679135, A679136, A679137, A679138,	spotted bowerbird	5	x	x				x			x	x
Ch. nuchalis	N279106, N405909, A810986, A679251, A679237	great bowerbird	5	x	x				x				x
Prionodura newtoniania	N279343, N279344, A679496, A679497, A679506, A679447	golden bowerbird	6	x	x	x			x		x		
Ptilonorhynchus violaceus			5			x			x	x	x	x	
Pt. v. minor	N279345, N15104, A679421	satin bowerbird	3			x			x	x		x	
Pt. v. violaceus	N278100, N278096		2						x	x	x	x	
Scenopoeetes dentirostris	N121199, N131198	tooth-billed bowerbird	2	x					x				
Sericulus ardens	A427631, A427632	flame bowerbird	2		x	x	x			x		x	
Se. aureus	N145532, A679287, A303007	masked bowerbird	3	x	x	x	x			x		x	x
Se. bakeri	A679305, A791268	Adelbert bowerbird	2		x	x							x
Se. chrysocephalus	N189646, N121226, A679320, A679332	regent bowerbird	4		x	x		x	x				x

Table 7

Order of gene regions	Coding sequence: Terminal regions	Coding sequence: TMRs	Coding sequence: Loops	Coding sequence: Other regions	**Introns**	Length (base pairs)	dS rate	dN rate	Overall rate
	N-terminal					60	0.050	0.000	0.050
		H-I				90	0.033	0.011	0.044
			I-1			18	0.000	0.000	0.000
		H-II				90	0.000	0.000	0.000
			E-1			18	0.056	0.056	0.111
		H-IIIa				45	0.044	0.000	0.044
					1	587	-	-	0.114
		H-IIIb				54	0.019	0.000	0.019
			I-2			33	0.000	0.000	0.000
		H-IV				69	0.072	0.000	0.072
			E-2a			18	0.000	0.000	0.000
					2	330	-	-	0.112
			E-2b			60	0.017	0.000	0.017
		H-V				27	0.000	0.000	0.000
			I-3			63	0.000	0.000	0.000
		H-VI				81	0.012	0.000	0.012
			E-3			24	0.000	0.000	0.000
		H-VII				63	0.000	0.000	0.000
				unlabeled		6	0.000	0.000	0.000
				H-VIIIa		6	0.000	0.000	0.000
					4	537	-	-	0.153
				H-VIIIb		27	0.000	0.000	0.000
	C-terminal					60	0.083	0.000	0.083
Terminal regions						120	0.067	0.000	0.067
TMRs						519	0.023	0.002	0.025
Loops						234	0.009	0.004	0.013
Other regions						39	0.000	0.000	0.000
Total coding sequence						912	0.020	0.000	0.030
Introns						1454			0.128

Figure captions

Figure 5- SWS1 opsin gene showing labeled regions (N- N-terminal region, H- helix, I- intercellular loop, E- extracellular loop, box- intron, C- C-terminal region) and primer locations; length of gene regions are not to scale; grey area represents missing sequence which includes the exon three. Primers and length of aligned sequences can be found in appendix H

Figure 6- Bowerbird phylogeny (see Chapter 1) on which maximum UV reflectance between 300 and 400 nm (as a percentage relative to the white standard), amino acid residues at positions 86 and 90, and habitat (Kusmierski et al. 1997, Beehler et al. 1986, Simpson & Day 2004, Frith & Frith 2004) are mapped

Figure 7- Selected spectra showing percent reflectance, in relation to a white standard of 100%, of yellow to red colored patches from male forest species showing characteristic peaks in the UV, potentially contributable to carotenoid pigmentation; Am.macg- *Amblyornis macgregoriae*, Am.suba- *Am. subalaris*, Pr.newt- *Prionodura newtoniania*, Se.arde- *Sericulus ardens*, Se.aure- *Se. aureus*, Se.bake- *Se. bakeri*, Se.chry- *Se. chrysocephalus*

Figure 5

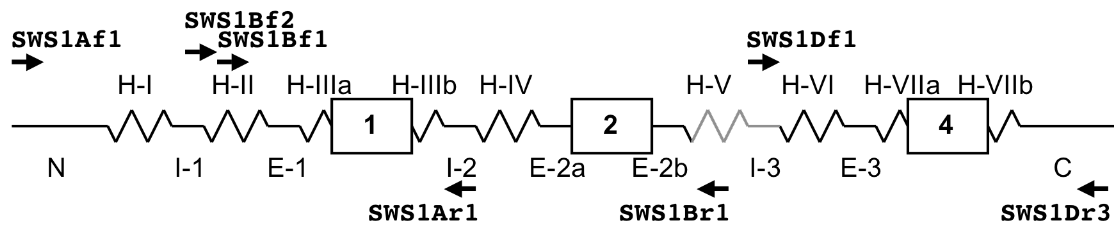


Figure 6

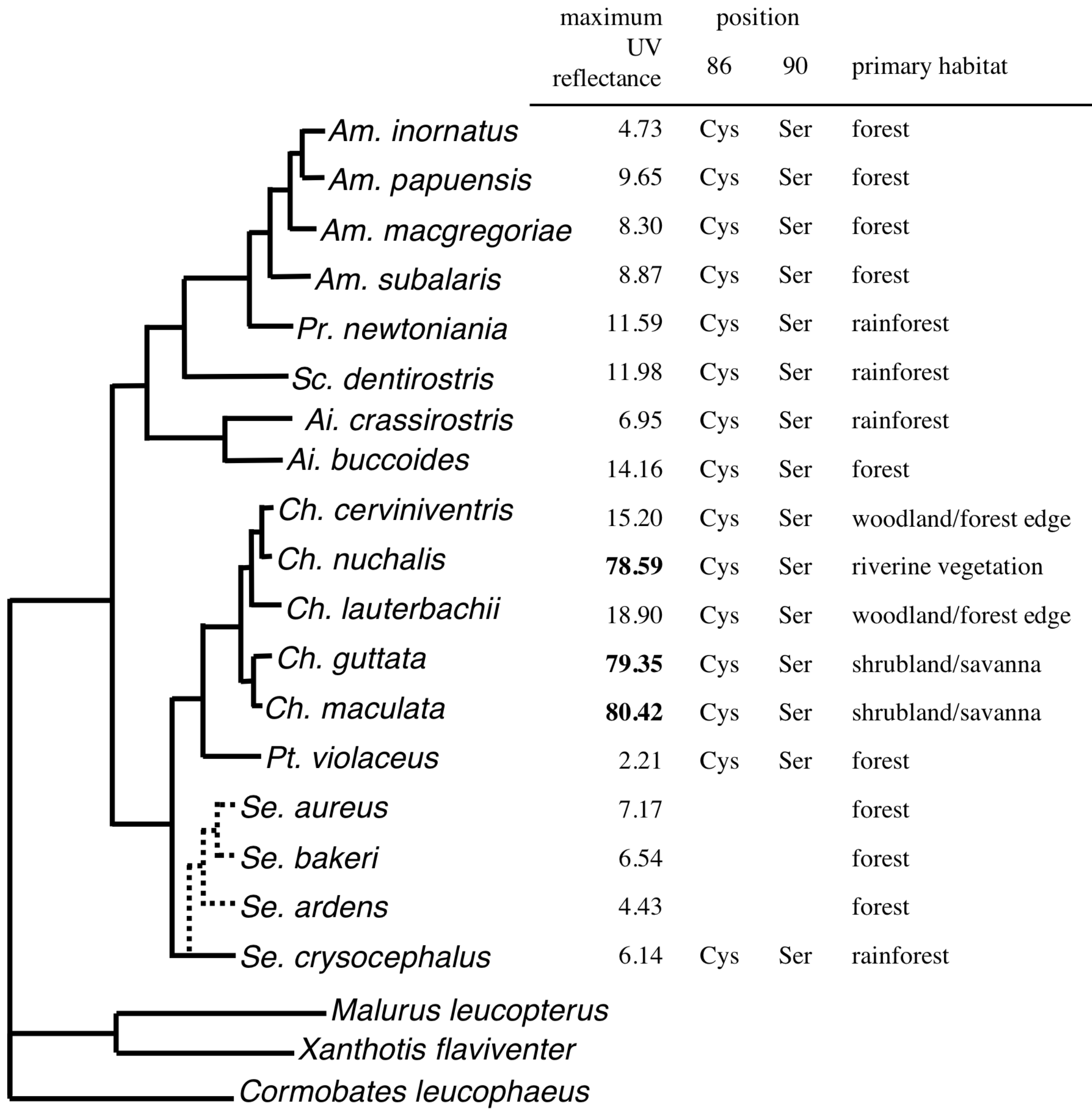
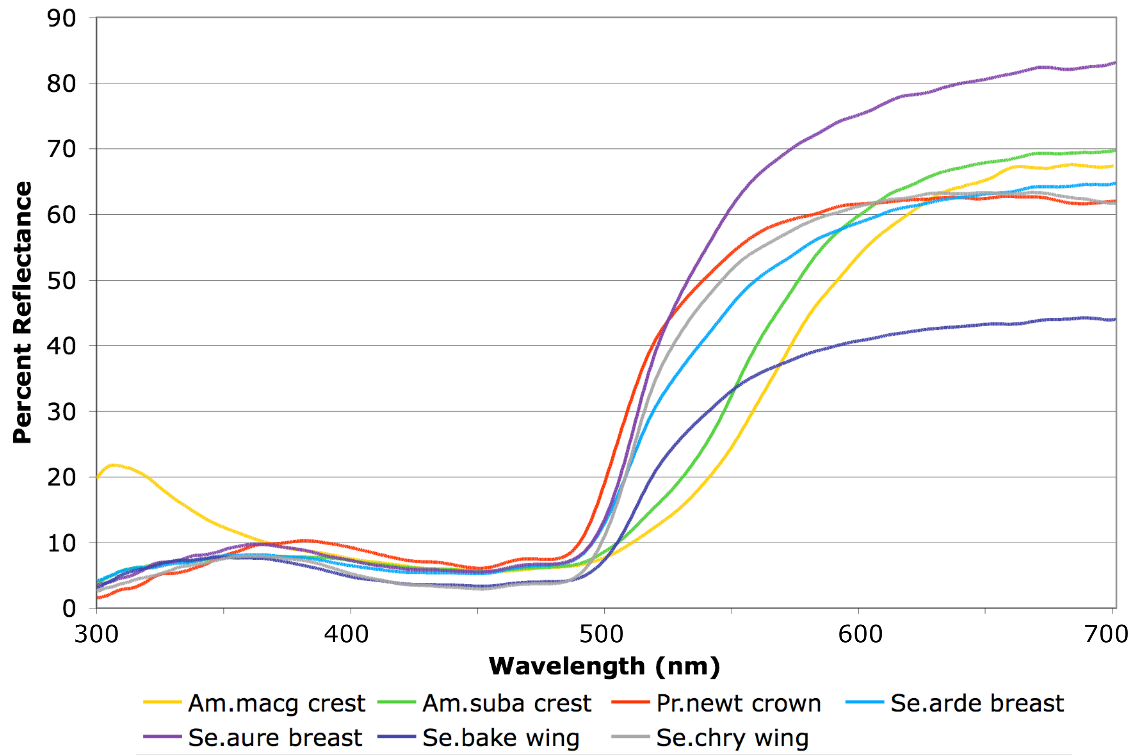


Figure 7



CHAPTER IV

A key amino acid change in the Passeriform SWS1 opsin gene accompanies near simultaneous changes at other positions and matches plumage UV reflectance

Abstract

The short-wavelength-sensitive 1 (SWS1) opsin gene codes for a UV sensitive opsin pigment, where a few key amino acid replacements have been shown to cause significant shifts in UV pigment sensitivity, and potentially affect UV vision. Large shifts in UV pigment spectral sensitivity found within avian species are associated with substitutions at position 90. To better understand how the SWS1 gene evolves within the Passeriformes (perching birds), I sequenced a region of the SWS1 gene that includes several key positions from 134 species representing 79 genera. I show that 1) only two replacements have occurred at position 90 across the passerines, a serine to cysteine change in the basal oscines, and a reversion to serine in the basal "core Corvoidea", and 2) associated with the two replacements at position 90 are nearly simultaneous replacements at four other positions that are otherwise rare. This curious pattern suggests a constraint on replacements at position 90, perhaps due to the need for coordinated conformational changes associated with the retinal binding pocket, that have limited adaptation in the UV visual system. Additionally, since UV vision has been predicted to match UV reflectance, I test whether replacements at position 90 associate with differences in intensity of UV reflectance from plumage measured from 91 of the SWS1

sequenced species. My results follow predictions where species with short UV wavelength shifted pigments reflect more intensely in shorter UV wavelengths (300 – 350 nm) than species with long UV wavelength shifted pigments. However, these results must be considered with caution due to a lack of independence caused by constraints on SWS1 evolution.

Introduction

Several studies (e.g. Andersson & Amundsen 1997, Bennett et al. 1996, Bennett et al. 1997, Hunt et al. 1999, Siitari et al. 2002) have suggested that vision in the ultraviolet range is important in avian communication. Because of this, considerable attention has been paid to factors that may influence UV visual abilities. Specifically, amino acid replacements at key positions, particularly within the retinal binding pocket (RBP; Carleton et al. 2005), in the short-wavelength-sensitive 1 (SWS1) opsin pigment have been shown to shift the spectral sensitivity of the UV pigment more than 30 nanometers (nm), presumably resulting in changes to the UV visual system. Evidence from studies using site-directed mutagenesis, and from a few naturally occurring pigments, have shown that cysteine at positions 86 or 90 (Cys86 or Cys90) result in UV pigment sensitivity shifted towards shorter UV wavelengths, with a wavelength of maximum absorption (λ_{max}) around 370 nm; serine at these positions (Ser86 or Ser90) result in UV pigment sensitivity shifted towards longer UV wavelengths and a λ_{max} around 405 nm (Yokoyama et al. 2000, Wilkie et al. 2000, Shi et al. 2003, Carvalho et al. 2007). It is unclear though, how replacements at SWS1 positions evolve within specific, closely related avian groups (but see Chapter 3), since most studies focus either within

individual species (see Yokoyama et al. 2000), or among several but distantly related species (see Ödeen & Håstad 2003). By sequencing a region of the SWS1 opsin gene containing key (i.e. positions 86 and 90) and nearby positions from 134 passeriform species and mapping these replacements onto recently estimated passeriform molecular phylogenies (Barker et al. 2004, Irestedt and Ohlson 2008), I am able to evaluate the evolution of replacements associated with the RBP in the SWS1 gene. In addition, mapping changes at positions near 86 and 90 may allow for a better understanding of the evolution of shifts in UV sensitivity.

Because UV sensitivity is difficult to directly assess, UV plumage reflectance has been used to infer the ability to see and use UV signals (Hausmann et al. 2003). A considerable amount of research has shown that UV wavelengths are commonly reflected from the plumage of many avian species (Eaton & Lanyon 2003, Eaton 2005, and Mullen 2007). Yet, the lack of information describing UV visual abilities (e.g. SWS1 sequences) from many species for which measurements of UV reflection are also available, has not allowed for a test of the assumption that UV reflectance is correlated with UV sensitivity (see Mullen and Pohland 2008). Using SWS1 amino acid sequences and measurements of UV reflectance taken from the plumage of 91 passeriform species, I tested whether UV sensitivity, inferred from replacements at key sites within the SWS1 pigment, associate with UV reflectance. Specifically, since Cys90 results in pigments with a sensitivity shifted towards shorter UV wavelengths, I ask whether species with Cys90 pigments will reflect more intensely in shorter UV wavelengths where Ser90 pigments are not sensitive, but not in longer UV wavelength where both Cys90 and Ser90 pigments are sensitive. By focusing within a single group of avian species, I am able to address in detail both the

manner in which SWS1 amino acid sequences evolve, and whether replacements at key SWS1 amino acid positions associate with changes in plumage UV reflectance within the Passeriformes.

Methods

To characterize variation within the passeriform SWS1 opsin sequence, and using the Barker et al. (2004) passeriform phylogeny to guide my selection of species, I sequenced (or collected from GenBank; see appendix I) a region of the SWS1 opsin gene containing position 78 through 101 (bovine rhodopsin, Hargrave & McDowell 1992; Palczewski et al. 2000) from 134 passeriform species that representatively covered the topology. The sequenced region included positions 86 and 90, which have previously been shown to shift pigment sensitivity (Yokoyama et al. 2000, Wilkie et al. 2000, Shi et al. 2003, Carvalho et al. 2007) as well as nine other RBP positions (Carleton et al. 2005). I then mapped the amino acid sequences onto both the Barker et al. (2004; figures 8 (family level) and 10 (species level)) and Irestedt and Ohlson (2007; figure 11) passeriform phylogenies. To test for possible associations among SWS1 amino acid positions, I used Pearson correlation coefficients calculated for each position in relation to principle component analyses (PCA) factors, to test for significant correlations in the pattern of amino acid replacements among all variable positions.

Also, to test whether differences in UV visual abilities, estimated from replacements at key SWS1 positions, match variation in UV reflectance, I first measured intensity of UV reflectance from the plumage of 91 of the 134 sequenced species from preserved museum skins. Then using t-tests, I tested for significant differences in mean

UV reflectance intensity between groups defined by replacements at amino acid positions in 10 nm intervals from 300 to 400 nm. Detailed methods describing SWS1 amplification and sequencing, measurements of UV reflectance intensity, and statistical methods can be found in the supplemental material.

Results

To characterize the evolution of the SWS1 pigment, I began by describing the evolution of replacements at position 90, which have been shown to greatly influence pigment sensitivity. Inferred from nucleotide sequences, I find only serine and cysteine at position 90, residues associated with violet-biased and UV-biased vision, respectively, and for which there is strong and consistent support (Yokoyama et al. 2000, Wilkie et al. 2000, Shi et al. 2003, Carvalho et al. 2007). Mapping position 90 onto the Barker et al. (2004) passeriform phylogeny (figures 8 and 10) results in a highly conserved evolutionary pattern, where Ser90 is predicted to have changed to Cys90 just once in the basal oscine species, and reverted back to Ser90 just once in the basal "core Corvoidea". This nearly identical pattern is found at several other positions including two other RBP positions, 85 and 91, and two non-RBP positions, 81 and 84 (table 8, figures 8 and 10). When testing for correlations among all variable positions (positions 81, 84, 85, 86, 88, 90, 91, and 96) to factors calculated from a PCA, I find that factor one explains 67.6% of the variation was highly (and significantly) correlated with positions 81, 84, 85, 90, and 91 (average correlation coefficient 0.97, p-values for all correlations <0.0001). Positions 86, 88, and 96 were not highly correlated with factor one in this PCA. When testing for correlations between only variable RBP positions (positions 85, 86, 90, and 91) and

factors calculated from a PCA, factor one was highly correlated with positions 85, 90, and 91, explaining 76.3% of the variation (table 9), while factor 2 was highly correlated with only position 86 (for more details, see supplemental material). Mapping the correlated positions onto the Irestedt and Ohlson (2007) passeriform phylogeny reveals the same general pattern as found using the Barker et al. (2004) tree, plus one additional simultaneous change associated with the placement of *Pachycephalopsis* (figure 11). I discuss results associated with position 86 in the supplemental material because the effects of amino acid residues found at position 86 on UV pigment spectral sensitivity are either controversial (Shi et al. 2003, Carvalho et al. 2007) or unknown. These results show that amino acid residues at several positions, both within and in close proximity to the RBP are highly correlated with residues found at position 90.

Because UV visual abilities have been assumed to match UV reflectance, I tested the hypothesis that species with pigments predicted to have a greater sensitivity to shorter UV wavelengths (defined by Cys90) reflected more intensely in shorter UV wavelengths, then species with pigments predicted to be less sensitive to shorter UV wavelengths (ser90). The intensity of UV reflectance from the plumage of 91 passeriform species varied considerably, with a notable outlier *Pitta versicolor* (see supplemental material) that was removed prior to analysis. Results from the t-tests comparing mean UV reflectance intensity between groups of species defined by replacements at position 90 followed predictions where species with Cys90 reflected more intensely in shorter UV wavelengths (300 - 350 nm) than species with Ser90 pigments, but not in longer wavelengths (350 - 400 nm) where both pigments are predicted to be sensitive (figure 9).

Discussion

The lack of data comparing naturally occurring UV pigments among relatively closely related species has resulted in a poor understanding of how this pigment, and potentially the ability to see UV wavelengths, evolves in avian species. Characterization in this study of variation at SWS1 amino acid positions from 134 passeriform species reveals a strong correlation between residues identified at position 90 and residues at four other positions, found both within and in close proximity to the RBP. When mapped onto either of two alternative (but similar) phylogenies based on unlinked nuclear genes, the pattern implies that these positions remained largely static from the evolution of suboscine passeriforms through the basal oscines, then evolved nearly simultaneously at all five positions in oscine taxa just prior to the passeridan split, and then reversed to the earlier character states in the basal "core Corvoidea". No variation within these groups suggests that strong directional selection may explain this pattern. Oddly, there is no difference in codon sequences among the earlier and later groups. This could indicate that the "reversal" did not occur (i.e. is not the result of homoplasy) but reflects either that the passeriform topologies are incorrect, or there was sequence polymorphism that sorted non-parsimoniously during the period in which these basal changes occurred (see Degnan and Rosenberg 2009). These simultaneous changes are unprecedented in the SWS1 opsin literature, and, to my knowledge, in the evolution of any protein examined in a phylogenetic context across a clade of organisms.

The fact that these positions are so highly correlated and stereotyped is difficult to explain. It suggests though, that replacements at these positions, being that they are either within or closely associated with the RBP and hence important to protein conformation

and function, require coordinated changes at all positions resulting in a constraint on replacements at position 90. If so, a constraint on position 90, due to these required coordinated changes with other positions, may limit shifts in the spectral sensitivity of the SWS1 pigment, and because of the possible effects of SWS1 sensitivity on UV vision, constrain variability in the passeriform UV visual system. Evidence from several studies show that site-directed mutagenesis of single amino acid positions have large effects on the pigment's spectral sensitivity (Yokoyama et al. 2000, Wilkie et al. 2000, Shi et al. 2003, Carvalho et al. 2007) but do not involve coordinated changes at nearby positions. Yet through the analysis of natural SWS1 opsin genes, I can speculate that other positions in and near the RBP may affect UV vision by constraining replacements at position 90, possibly because they have a heretofore undetected effect on the binding of retinol to the RBP.

Previous papers have predicted associations between UV visual abilities and UV reflectance from plumage (see Hausmann et al. 2003), and results presented here support this prediction. It is also possible that phylogenetic effects (i.e. a lack of independence among phylogenetic groups) could explain the correlation I find, where three groups of birds defined by position 90 replacements show high within group similarity in UV reflectance. While there is reasonable evidence that plumage coloration is highly labile (Omland and Lanyon 2000, Zwiers et al. 2008, Chapter 2), suggesting an adaptive basis for an association between SWS1 replacements and UV reflectance, I cannot rule out the effects of phylogeny. My results also do not include the possible effects of other factors that may influence UV visual capabilities (e.g. ocular media transmission and UV cone density). Additional information on factors such as ocular media transmission within the

Passeriformes is needed to determine whether UV vision can accurately be estimated from SWS1 replacements.

In a recent paper, Mullen and Pohland (2008) found significant differences in the location of peak UV reflectance in relation to position 90 replacements. While the association I find between UV reflectance and SWS1 replacements is similar, our results differ in several important ways. Two key differences are how species are sampled and the amount of data available to characterize species' UV visual abilities. First, Mullen and Pohland (2008) use the SWS1 sequences of only 56 species (Ödeen & Håstad 2003), sampling few species per order, to infer the UV visual abilities of 968 species. I collected UV reflectance data and SWS1 sequences from each of 91 passeriform species, representing 79 genera and 48 families. Mullen and Pohland (2008) also assume that all species in the same order have the same UV visual system, even though evidence for mixed UV visual systems within orders had been reported, i.e. Ciconiiformes, Struthioniformes, and Passeriformes (Ödeen & Hästad 2003). By focusing within a specific order, and using SWS1 replacements to estimate UV visual abilities and measures of UV reflectance, my results provide stronger evidence that UV reflection matches SWS1 replacements, and potentially UV visual abilities, within the Passeriformes.

In the present study, comparisons across the Passeriformes suggest an association, albeit potentially limited by a lack of independence, between SWS1 replacements and UV plumage reflectance. This differs from my study in the bowerbirds (Chapter 3) where extreme difference in UV reflectance is associated with habitat but not with SWS1 replacements. In that study I found no variation at key and other nearby positions within the SWS1 RBP amino acid sequence, even though there is extreme variation in UV

plumage reflectance closely associated with differences in habitat among species of the same genus. I hypothesize that given the shorter timeframe of bowerbird evolution (relative to passeriform evolution), and the conservative nature of replacements at position 90 and associated sites, that UV reflectance (and use of UV signals) within smaller, more recently derived groups of species is, in the bowerbirds, not dependent on SWS1 replacements. The combined results of these chapters suggest an interesting interplay between constraints on SWS1 evolution and how adaptation occurs relative to differences in the UV light environment.

Table captions

Table 8- Differences in the pattern of amino acid replacements at each position compared to that found at position 90, using all species with data at the position of interest; positions grouped in the first factor of the principle component analyses (that included all variable positions) are in bold

Table 9- Pearson correlation coefficients and associated *p*-values (in italics) for positions in relation to factors calculated in two separate principle component analyses, the first including all variable positions, and the second including only variable positions within the retinal binding pocket (RBP); positions are labeled as either found within or outside the RBP

Table 8

Position	Number of species (species with missing data removed)	Found within the retinal-binding pocket?	Number of differences compared to position 90 replacement pattern	Percentage of differences compared to position 90 replacement pattern
81	**130**	**no**	**5**	**3.8**
82	128	no	72	56.3
83	132	no	75	56.8
84	**131**	**no**	**1**	**0.8**
85	**133**	**yes**	**11**	**8.3**
86	133	yes	55	41.4
88	132	no	28	21.2
91	**133**	**yes**	**3**	**2.3**
96	131	no	68	51.9

Table 9

Including all variable positions (n=84)

Position	Factor 1	Factor 2
% variation explained	67.6	14.4
81 (non-RBP)	0.96069	-0.08657
	<0.0001	*0.3410*
84 (non-RBP)	0.98319	-0.08192
	<0.0001	*0.3677*
85 (RBP)	0.92229	-0.05364
	<0.0001	*0.5557*
86 (RBP)	0.36145	-0.70336
	<0.0001	*<0.0001*
88 (non-RBP)	0.72378	0.05589
	<0.0001	*0.5392*
90 (RBP)	0.98319	-0.08192
	<0.0001	*0.3677*
91 (RBP)	0.98319	-0.08192
	<0.0001	*0.3677*
96 (non-RBP)	0.19347	0.81562
	0.0320	*<0.0001*

Including only variable RBP positions (n=90)

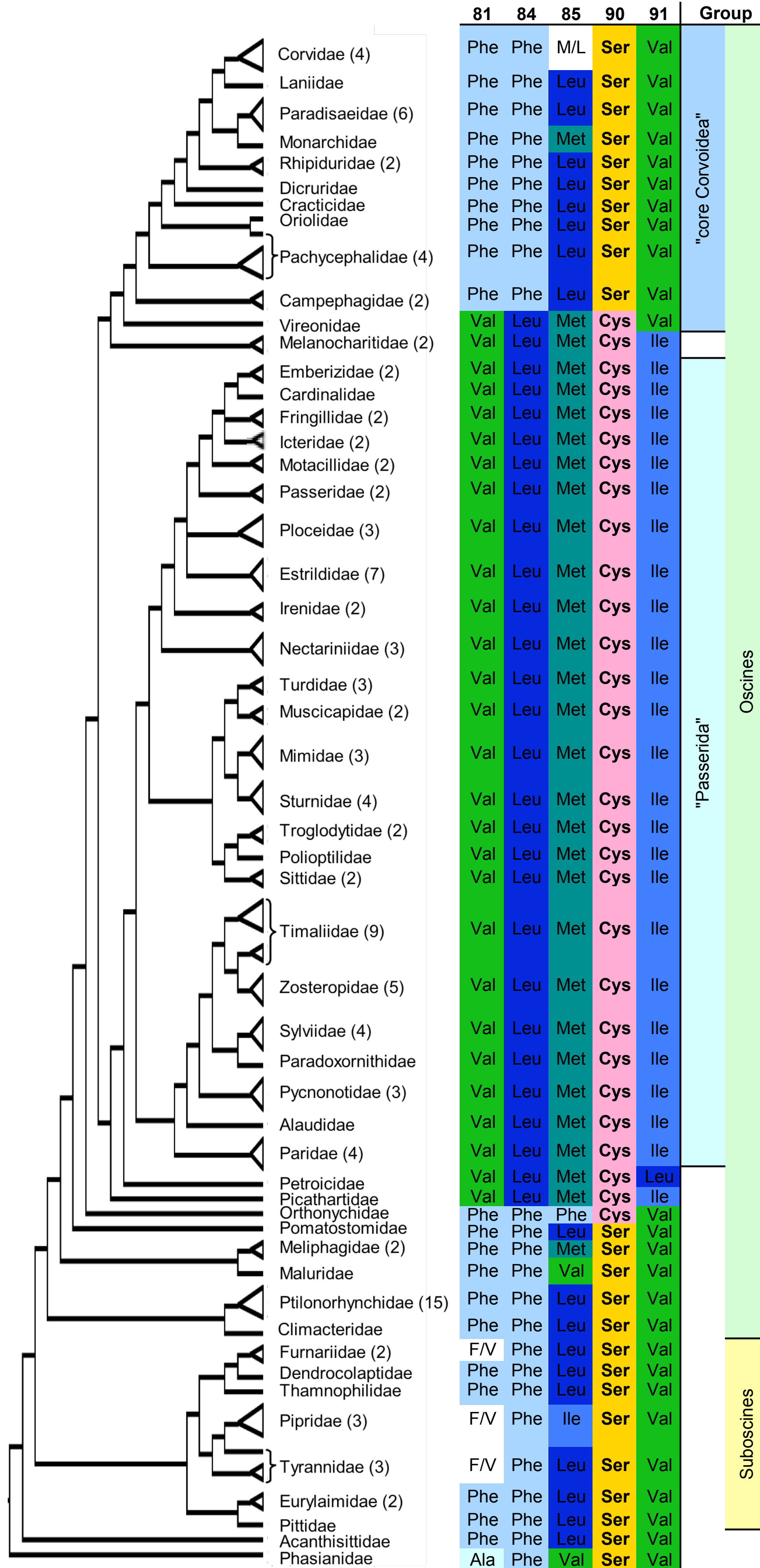
Position	Factor 1	Factor 2
% variation explained	76.3	19.5
85 (RBP)	0.92749	0.16341
	<0.0001	*0.0709*
86 (RBP)	0.20372	0.97889
	0.0238	*<0.0001*
90 (RBP)	0.96224	0.21739
	<0.0001	*0.0157*
91 (RBP)	0.96224	0.121739
	<0.0001	*0.0157*

Figure captions

Figure 8- Pattern of amino acid replacements showing near simultaneous changes at position 90 and four other positions when mapped onto the Barker et al. (2004) passeriform phylogeny, modified by removing families not included in my study; multiple residues at the same position within a family are reported using single letter amino acid abbreviations; number in parentheses after family name at ends of branches designates the number of species sampled within the family

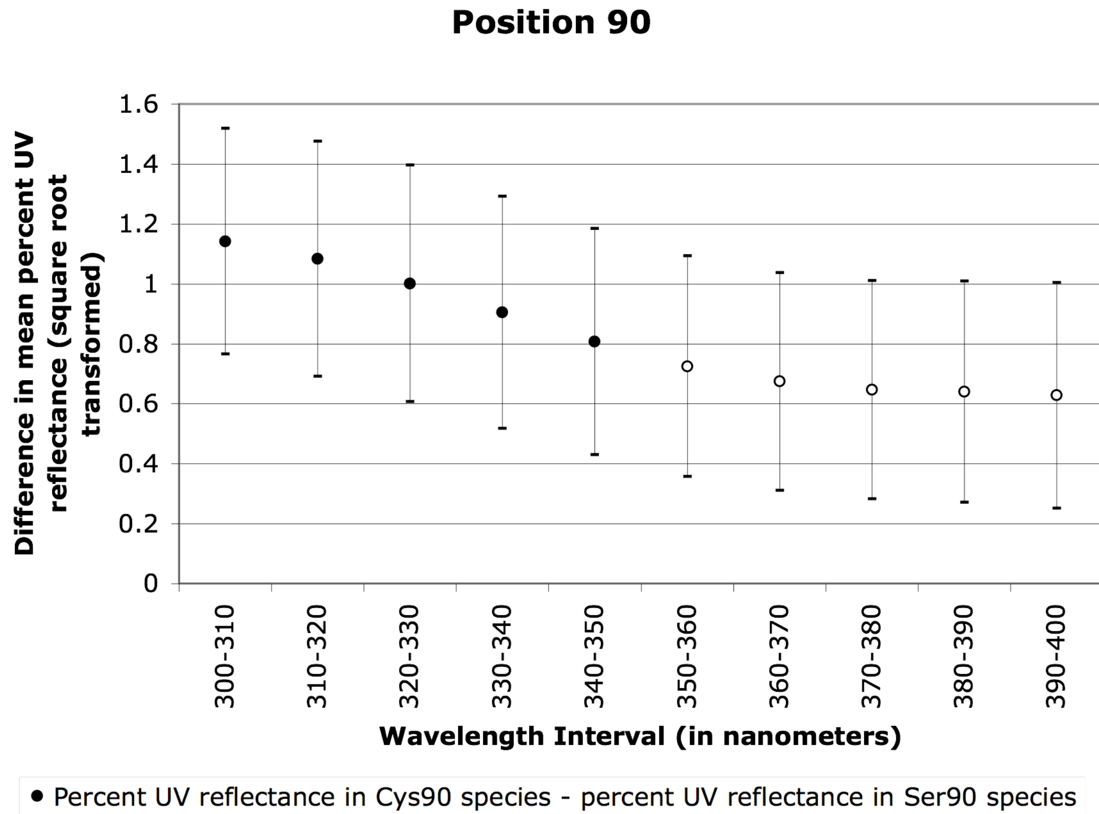
Figure 9- Difference in mean percent UV reflectance (square root transformed) between species with cysteine 90 (Cys90, n = 48) and serine 90 (Ser90, n = 42) after removing outlier (*Pitta versicolor*; see text); filled circles represent significant differences from 0 using an alpha of 0.05, p-values are two-tailed, bars indicate standard errors

Figure 8



Taxon	81	84	85	90	91
Corvidae (4)	Phe	Phe	M/L	Ser	Val
Laniidae	Phe	Phe	Leu	Ser	Val
Paradisaeidae (6)	Phe	Phe	Leu	Ser	Val
Monarchidae	Phe	Phe	Met	Ser	Val
Rhipiduridae (2)	Phe	Phe	Leu	Ser	Val
Dicruridae	Phe	Phe	Leu	Ser	Val
Cracticidae	Phe	Phe	Leu	Ser	Val
Oriolidae	Phe	Phe	Leu	Ser	Val
Pachycephalidae (4)	Phe	Phe	Leu	Ser	Val
Campephagidae (2)	Phe	Phe	Leu	Ser	Val
Vireonidae	Val	Leu	Met	Cys	Val
Melanocharitidae (2)	Val	Leu	Met	Cys	Ile
Emberizidae (2)	Val	Leu	Met	Cys	Ile
Cardinalidae	Val	Leu	Met	Cys	Ile
Fringillidae (2)	Val	Leu	Met	Cys	Ile
Icteridae (2)	Val	Leu	Met	Cys	Ile
Motacillidae (2)	Val	Leu	Met	Cys	Ile
Passeridae (2)	Val	Leu	Met	Cys	Ile
Ploceidae (3)	Val	Leu	Met	Cys	Ile
Estrildidae (7)	Val	Leu	Met	Cys	Ile
Irenidae (2)	Val	Leu	Met	Cys	Ile
Nectariniidae (3)	Val	Leu	Met	Cys	Ile
Turdidae (3)	Val	Leu	Met	Cys	Ile
Muscicapidae (2)	Val	Leu	Met	Cys	Ile
Mimidae (3)	Val	Leu	Met	Cys	Ile
Sturnidae (4)	Val	Leu	Met	Cys	Ile
Troglodytidae (2)	Val	Leu	Met	Cys	Ile
Polioptilidae	Val	Leu	Met	Cys	Ile
Sittidae (2)	Val	Leu	Met	Cys	Ile
Timaliidae (9)	Val	Leu	Met	Cys	Ile
Zosteropidae (5)	Val	Leu	Met	Cys	Ile
Sylviidae (4)	Val	Leu	Met	Cys	Ile
Paradoxornithidae	Val	Leu	Met	Cys	Ile
Pycnonotidae (3)	Val	Leu	Met	Cys	Ile
Alaudidae	Val	Leu	Met	Cys	Ile
Paridae (4)	Val	Leu	Met	Cys	Ile
Petroicidae	Val	Leu	Met	Cys	Leu
Picathartidae	Val	Leu	Met	Cys	Ile
Orthonychidae	Phe	Phe	Phe	Cys	Val
Pomatostomidae	Phe	Phe	Leu	Ser	Val
Meliphagidae (2)	Phe	Phe	Met	Ser	Val
Maluridae	Phe	Phe	Val	Ser	Val
Ptilonorhynchidae (15)	Phe	Phe	Leu	Ser	Val
Climacteridae	Phe	Phe	Leu	Ser	Val
Furnariidae (2)	F/V	Phe	Leu	Ser	Val
Dendrocolaptidae	Phe	Phe	Leu	Ser	Val
Thamnophilidae	Phe	Phe	Leu	Ser	Val
Pipridae (3)	F/V	Phe	Ile	Ser	Val
Tyrannidae (3)	F/V	Phe	Leu	Ser	Val
Eurylaimidae (2)	Phe	Phe	Leu	Ser	Val
Pittidae	Phe	Phe	Leu	Ser	Val
Acanthisittidae	Phe	Phe	Leu	Ser	Val
Phasianidae	Ala	Phe	Val	Ser	Val

Figure 9



Chapter IV Supplemental Material

Short-wavelength-sensitive 1 sequencing

Amplification of the SWS1 transmembrane region 2 was done for 134 species following methods described in Ödeen & Hästad (2003) using the SU193a/SU306b primer set. In several species, gene regions matching the medium-wavelength-sensitive opsin pigment, coded for by the rhodopsin 2 gene, were amplified. Therefore, I designed internal primers (SU193+17: TGA ACA TCT CCK TCA GCG and SU306-14: AAG TAT CCC TGG GMR CTG) to specify SWS1 TMR2 amplification. Cycle sequencing reactions were done using BigDye v 3.1 terminator mix, following ABI protocols but in 1/8th reactions, and analyzed on an ABI 3100 automated sequencer. DNA sequences were aligned in Sequencher 4.7 (Gene Codes, Ann Arbor), checked for anomalies, and translated to amino acids using the universal code in MacClade 4.06 (Maddison & Maddison). Amino acid residues between positions 78 and 101 for each species where UV reflectance measurements were made, and for the additional species without UV reflectance data, can be found in figure 10. Due to the use of redesigned primers, data from positions 78 and 79, and 97 through 101 are missing from approximately a third of the species. Therefore, statistical analyses were performed using positions 80 through 96. Some may argue that the simultaneous changes found at five amino acid positions in the SWS1 pigment stems from the biased amplification of two or more copies of the SWS1 gene. I believe this is unlikely since I used two different primer sets to amplify and sequence the targeted gene region; I might have expected to find nucleotide ambiguities if multiple genes were being amplified, yet I found none and I found no association

between primer set and replacements. Also, when summarizing the available physiological evidence for UV vision in passeriform species, I found a substantial sampling bias, where most species whose UV visual system had been tested exhibited a cysteine at positions 86 and 90 (table 10). Therefore, I suggest a stronger emphasis should be placed on collected additional physiological evidence for UV visual abilities from non-Cys86 and Cys90 species.

Measuring intensity of UV reflectance

Measurements of UV reflectance intensity were made from the preserved skins of males of 91 passeriform species, located in the collections of the U. S. National Museum (Smithsonian Institution) and the American Museum of Natural History. A detailed list of all specimens is in appendix I. Measurements of UV reflectance followed previously published methods (Chapter 3). Briefly, using an Ocean Optics S2000 spectrometer and PTFE as a white reference, each skin was fully scanned to reveal any UV reflecting patches, defined as a region of plumage with continuous coloration defined by body regions, i.e. uniform spectra over crown and nuchal crest were treated as two separate patches. Care was taken not to include underplumage or patches on the underside of the body, as these areas are typically white or near white and may contain UV reflecting elements (Eaton & Lanyon 2003; Eaton 2005) but which are not likely used in signaling (Hausmann et al. 2003). I made three spectral measurements of each UV reflecting patch on each individual, recording reflectance intensity every 35 hundredths of a nanometer. I averaged reflectance values across the three measurements and smoothed over 10 nm in each direction, with the closest wavelengths contributing proportionally more to the

overall value. Intensity of UV reflectance for each patch was calculated in 10 nm intervals from 300 to 400 nm for each patch (see Siitari et al. 2007). Since the number of measured patches was different among species, maximum rather than average percent UV reflectance intensity, relative to the white standard, is reported for each 10 nm interval.

Statistical analyses

To test for similarity in the pattern of replacements at variable amino acid positions, I tested for correlations among the positions using principle component analyses (PCA). Residues at each position were scored in an ordinal fashion starting at the basal passeriform species *Acanthisittidae* (i.e. for position 90, serine = 0, cysteine = 1; the outgroup *Gallus gallus* was excluded from all PCAs). Six species (*Corvus monedula*, *Dumetella carolinensi*s, *Lamprotornis superbus*, *Yuhina nigrimenta*, *Manacus manacus*, and *Vireo griseus*) with missing or ambiguous data at one or more positions between 80 and 96 were removed prior to an initial PCA analysis that included all other species and all variable amino acid positions between 80 and 96 (i.e. positions 81-86, 88, 90, 91, and 96). From this initial analysis, I found that four species with unique replacements (*Tityra semifasciata* (Tyr86), *Orthonyx spaldingii* (Phe85), *Malurus leucopterus* (Val85), and *Tregellasia capito* (Leu91)), and two positions with missing or unique replacements in four or fewer species that would otherwise be non-variable (position 82- unique replacements in two genera (four species total), two species with missing data; position 83- unique replacement in one species), greatly influenced how the PCA factors were calculated and were removed from further analyses. Removing outlying data in this

manner allowed the retention of the most amount of data (number of taxa = 123) in the final PCA analyses (i.e. removing position 83 with only one replacement in *Gymnorhina tibicen*, instead of removing the species entirely, retained data from this species for other positions and excluded an otherwise non-variable position; excluding *Tityra semifasciata* because of its unique replacement at position 86, instead of removing the entire position, retained the variable data found at this position for other species).

Using these data, I performed two PCAs, one including all variable positions and a second including only variable positions within the RBP. In the first PCA including all variable positions (positions 81, 84, 85, 86, 88, 90, 91, and 96), factor one explained 67.6% of the variation with an eigenvalue of 5.41, and contained five positions (81, 84, 85, 90, and 91) that were all highly correlated to factor one (average Pearson correlation coefficient, 0.97) and were highly significant (p-values, <0.0001; table 9). While significant at at least a 0.05 level for factor one, the remaining three positions were in comparison not strongly correlated with factor one (position 86, 0.36; position 88, 0.72; position 96, 0.19). The second factor in this analysis explained 14.4% of the variation with an eigenvalue of 1.15, and shows an orthogonal relationship between positions 86 (correlation coefficient -0.70, p-value <0.0001) and 96 (correlation coefficient 0.82, p-value <0.0001). These results show 1) that positions 81, 84, 85, 90 and 91 all share a near identical pattern in amino acid replacements, 2) replacement patterns found at positions 86 and 88 are not as similar compared to the highly correlated positions (i.e. 81, 84, 85, 90, and 91), and 3) the pattern of amino acid replacements at position 96 is unlike that found at all other variable positions.

To investigate correlations among only RBP positions, I ran a second PCA that only included variable positions in the RBP (positions 85, 86, 90, and 91). The analysis by default returned only one factor, which explained 76.3% of the variation (eigenvalue of 3.05) and contained highly significant proportions (p-values, <0.0001) of positions 85, 90, and 91 with an average Pearson correlation coefficient to factor one of 0.95 (table 9). Position 86 was also significant (p-value, 0.02), but had a correlation coefficient of 0.20. Forcing the retention of the second factor (eigenvalue of 0.78) revealed that it explained 19.5% of the variation, contained a highly significant proportion of position 86 (correlation coefficient 0.98, p-value <0.0001), and significant proportions of position 90 and 91 (p-value 0.02) but with very low correlation coefficients (0.22). Results from this analysis show that the pattern of amino acid residues among positions 85, 90 and 91 is extremely similar, and very different from that found at position 86.

I wanted to test whether differences in UV vision, defined by replacements at key positions (i.e. positions 86 and 90) within the SWS1 opsin pigment, match maximum UV reflection intensity measured from plumage. To do this, I compared the difference in maximum UV reflectance between groups of species defined by replacements at either position 86 or 90 using t-tests. Before the analysis, a square root transformation of the reflection data was necessary to normalize the data. After inspecting the transformed data I found that *Pitta versicolor* is an extreme outlier among other Ser90 species (average reflectance from 300 – 400 nm including all Ser90 species, 2.62%; average reflectance from 300 – 400 nm in Pi. versicolor, 9.41%; figure 10) and I removed it from analyses of both positions 86 and 90. Additionally, because *Manacus manacus* and *Tityra semifasciata* show unique replacements at position 86, these species were excluded only

from the position 86 analysis. This left a total of 88 species in the position 86 analysis and 90 species in the position 90 analysis. Means of the transformed maximum UV reflectance intensity data were compared among groups of species defined by amino acid residues at position 86, and separately at position 90, in 10 nm intervals between 300 and 400 nm. Significant differences in maximum UV reflection intensity were determined using an alpha of 0.05 and *p*-values are reported as two-tailed.

Position 86

I find that most species exhibit a cysteine at position 86 (Cys86), 24 species within the "Passerida" show a methionine (Met86), and one species each show serine (Ser86) and tyrosine (Tyr86) (*Manacus manacus* and *Tityra semifasciata* respectively), which were removed from analyses testing for significant difference in UV reflectance based on position 86 replacements. There is conflicting evidence supporting the effects of Cys86 on SWS1 sensitivity. Shi et al. (2003), using site-directed mutagenesis, suggest replacements of cysteine and serine at position 86 can result in shifts in SWS1 sensitivity comparable to position 90. On the other hand, Carvalho et al. (2007), analyzing pigments from wild-caught birds, show the common cormorant has Cys86 (and Ser90) but a SWS1 pigment with a maximum wavelength sensitivity at 405 nm. While the effects of Tyr86 are unknown, the only evidence for the effects of Met86 comes from *Lieothrix lutea*, which has the shortest wavelengths shifted SWS1 pigment so far measured in the Passeriformes (see table 10; Maier & Bowmaker 1993), yet a recent paper questions this finding (Hart & Hunt 2007). When intensity of UV reflectance was compared between Cys86 and Met86 species, Met86 was associated with significantly more intense UV

reflectance across the full 300 to 400 nm range in each interval (figure 12). Increased UV reflectance may be associated with increased UV sensitivity conferred by Met86. On the other hand, the significant difference in UV reflectance may result from the mitigation of reduced UV sensitivity resulting from Met86. Further research is required to more fully understand the effect Met86 has on SWS1 sensitivity and possibly overall UV visual abilities.

Table caption

Table 10- Evidence for UV vision in passeriform species from electroretinography (ERG), microspectrophotometry (MSP), and behavioral studies showing sampling bias, where most species are Cys86 and Cys90 and few species show alternative residues (e.g. Met86 and Ser90); UVS- ultraviolet sensitive, NUV- near ultraviolet sensitive, * indicates possible measurement error (see Hart and Hunt 2007); references: 1- Bowmaker et al. (1997), 2- Chen & Goldsmith (1986), 3- Das et al. (1999), 4- Hart et al. (1998), 5- Hart et al. (2000a), 6- Hart et al. (2000b), 7- Maier & Bowmaker (1993), 8- Parrish et al. (1984), 9- Yokoyama et al. (2000), 10- Das et al. (1999), 11- Maier (1992), 12- Probst et al. (2002), 13- Anderrson and Amundsen (1997), 14- Bennett et al. (1997), 15- Bennet et al. (1996), 16- Johnsen et al. (1998), 17- Andersson et al. (1998), 18- Maier (1993), 19- Siitari et al. (1999), 20- Siitari et al. (2002), 21- Siefferman (2005), 22- Pryke (2006)

Table 10

Species	Family	86	90	ERG	MSP	Behavior
Cyanocitta cristata	Corvidae	Cys	Ser	~370[2]		NUV[8]
Lanius excubitor	Laniidae	Cys	Ser			Foraging (visualization of scent marks)[12]
Melospiza melodia	Emberizidae	Cys	Cys	~370[2]		
Zonotrichia albicollis	Emberizidae	Cys	Cys	~370[2]		NUV[8] (*Z. querula* & *leucophrys*)
Cardinalis cardinalis	Cardinalidae	Cys	Cys	~370[2]		NUV[8]
Carpodicus mexicanus	Fringillidae	Cys	Cys	~370[2]	369[3]	
Serinus canaris	Fringillidae	Cys	Cys		366[10]	
Molothrus ater	Icteridae	Cys	Cys			NUV[8]
Passer domesticus	Passeridae	Cys	Cys	~370[2]		NUV[8]
Taeniopygia guttata	Estrildidae	Cys	Cys		360-380[1], 359[9]	mate choice[15]
Lonchura punctulata	Estrildidae	Cys	Cys		373[6] (*L. maja*)	male competition (*Erythrura goldiae*)[22]
Neochmia temporalis	Estrildidae	Cys	Cys		372[6] (*N. modesta*)	
Hylocichla mustelina	Turdidae	Cys	Cys	~370[2]		male competition (*Sialia sialis*)[21]
Turdus migratorius	Turdidae	Cys	Cys	~370[2]	373[5] (*T. merula*)	foraging (*T. iliacus*)[19]
Luscinia calliope	Muscicapidae	Cys	Cys			mate choice (*Ficedula hypoleuca*)[20]
Luscinia svecica	Muscicapidae	Cys	Cys			UVS[13], mate choice[16]
Dumetella carolinensis	Mimidae	Cys	Cys	~370[2]		
Mimus polyglottos	Mimidae	Cys	Cys	~370[2] (*Toxostoma rufum*)		
Sturnus vulgaris	Sturnidae	Cys	Cys		362[4]	NUV[8], mate choice[14]
Leiothrix lutea	Timaliidae	Met	Cys		355[7,11,*]	mate choice[18]
Parus atricapillus	Paridae	Cys	Cys	~370[2]		
Parus caeruleus	Paridae	Cys	Cys		372[5]	UVS[8], assortative mating[17]

Figure captions

Figure 10- Short-wavelength-sensitive 1 (SWS1) amino acid residues from positions 78 to 101 mapped onto the modified Barker et al. (2004) phylogeny, where ** represents the same species used by Barker et al. (2004), * represents the same genus, and where additional species from within the same family are included as a polytomy; residues are reported using three letter abbreviations, ambiguities are reported using single letter abbreviations; colored branches in the topology indicate maximum percent UV reflectance measured from plumage relative to the white standard (see legend)

Figure 11- Short-wavelength-sensitive 1 (SWS1) amino acid residues from positions 78 to 101 mapped onto the Irestedt & Ohlson (2007) phylogeny; placement of *Pachycephalopsis* results in an additional simultaneous change, in relation to trace based on the Barker et al (2004) phylogeny, at the five colored positions; "core Corvoidea" and "Passerida" taxa are represented by consensus sequences

Figure 12- Difference in mean percent UV reflectance (square root transformed) between species with methionine 86 (Met86, n = 9) and cysteine 90 (Cys90, n = 79) after removing *Pitta versicolor* (an extreme outlier; see text) and species with unique replacements at position 86 (see *Manacus manacus* and *Tityra semifasciata*; figure 1); filled circles represent significant differences from 0 using an alpha of 0.05, p-values are two-tailed, bars indicate standard errors

Figure 10

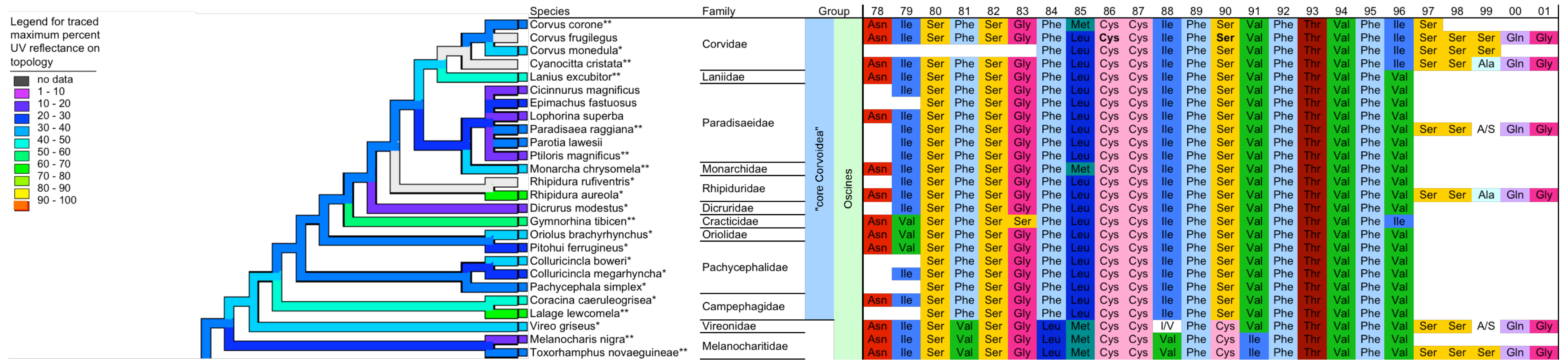


Figure 10 (cont'd)

Legend for traced maximum percent UV reflectance on topology

- no data
- 1 - 10
- 10 - 20
- 20 - 30
- 30 - 40
- 40 - 50
- 50 - 60
- 60 - 70
- 70 - 80
- 80 - 90
- 90 - 100

Group: "Passerida"; Oscines

Species	Family	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	00	01
Melospiza melodia	Emberizidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Zonotrichia albicollis		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Cardinalis cardinalis**	Cardinalidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Carpodicus mexicanus	Fringillidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Serinus canaris		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Dolichonyx oryzivorus	Icteridae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	**Cys**	Cys	Val	Phe	**Cys**	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Molothrus ater		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Motacilla lugens*	Motacillidae		Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Motacilla alba*			Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Passer montanus**	Passeridae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ala	Gln	Gly
Passer domesticus*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ala	Gln	Gly
Euplectes afer	Ploceidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Euplectes orix		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Ploceus nigricollis*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Amadina fasciata	Estrildidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	**Cys**	Cys	Val	Phe	**Cys**	Ile	Phe	Thr	Val	Phe	Val	Ala				
Chloebia gouldiae		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	**Cys**	Cys	Val	Phe	**Cys**	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Lonchura maja		Asn	Ile	Ser	Val	Ala	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ala	Ser	Ser	Gln	Gly
Lonchura punctulata		Asn	Ile	Ser	Val	Ala	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile					
Neochmia modesta		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ala	Ser	Ser	Gln	Gly
Neochmia temporalis			Ile	Ser	Val		Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ala	Ser	A/S	Gln	Gly
Taeniopygia guttata		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	**Cys**	Cys	Val	Phe	**Cys**	Ile	Phe	Thr	Val	Phe	Ile	Ala	Ser	Ser	Gln	Gly
Chloropsis aurifrons*	Irenidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Irena cyanogaster**				Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Nectarinia olivacea**	Nectariniidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Nectarinia chloropygia*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ala	Ser	Ser	Gln	Gly
Nectarinia aspasia*			Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Hylocichla mustelina	Turdidae		Ile	Ser	Val		Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	A/S	Gln	Gly
Turdus merula		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	**Cys**	Cys	Val	Phe	**Cys**	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Turdus migratorius*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Luscinia calliope	Muscicapidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Luscinia svecica		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Dumetella carolinensis	Mimidae								Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Mimus polyglottos*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Oreoscoptes montanus		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Acridotheres tristis	Sturnidae			Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Aplonis metallica		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile					
Lamprotornis superbus									Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Sturnus vulgaris**		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser			
Thryothorus ludovicianus	Troglodytidae			Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ala	Ser	Ser	Gln	Gly
Troglodytes aedon**		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ala	Ser	Ser	Gln	Gly
Polioptila dumicola*	Polioptilidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ser	Ser	Ser	Gln	Gly
Sitta carolinensis**	Sittidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ser	Ser	Ser	Gln	Gly
Sitta castanea*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Ile	Ser	Ser	Ala	Gln	Gly
Garrulax leucolophus*	Timaliidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Garrulax erythrocephalus*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Garrulax monilegar*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Garrulax nuchalis*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Garrulax pectoralis*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Leiothrix lutea		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Leiothrix argentauris		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Yuhina nigrimenta		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val			Ser	Ser	Ser	Gln	Gly
Yuhina bakeri		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Zosterops senegalensis**	Zosteropidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Zosterops erythropleurus*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Zosterops japonicus*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Zosterops montana*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Zosterops palpebrosus*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Phylloscopus trochilus	Sylviidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Phylloscopus reguloides		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Phylloscopus inornatus		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Phylloscopus trivirgatus		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Paradoxornis webbianus	Paradoxornithidae		Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Pycnonotus melanicterus*	Pycnonotidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ala	Gln	Gly
Pycnonotus cafer*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ala	Gln	Gly
Pycnonotus latirostris*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser		Gln	Gly
Alauda arvensis**	Alaudidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser		Gln	Gly
Parus atricapillus*	Paridae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
Parus caeruleus*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Parus major**		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Parus palustris*		Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Tregellasia capito*	Petroicidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Ile	Phe	Cys	Leu	Phe	Thr	Val	Phe	Ile	Ser	Ser	Ser	Gln	Gly
Picathartes gymnocephalus**	Picathartidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly

Figure 10 (cont'd)

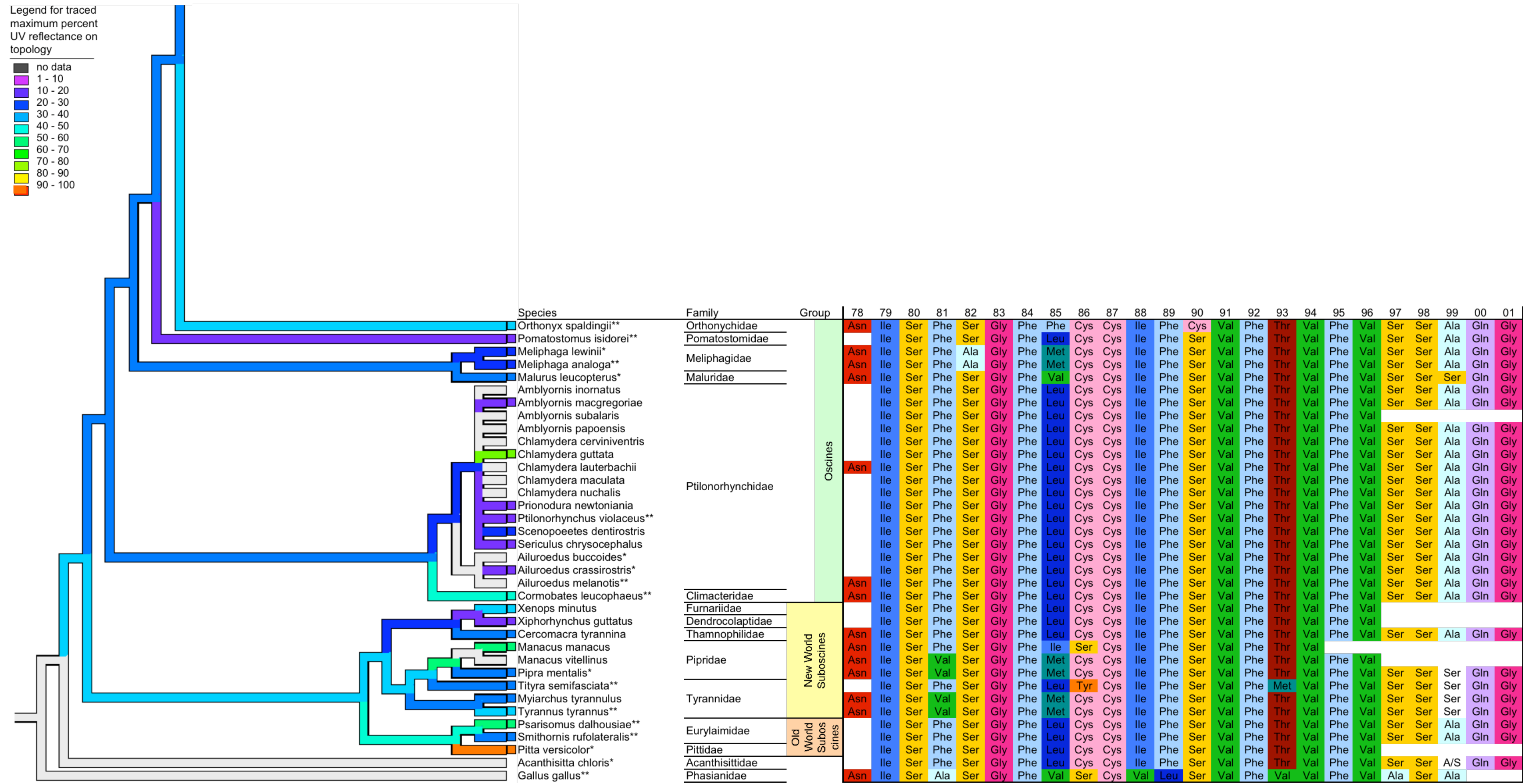
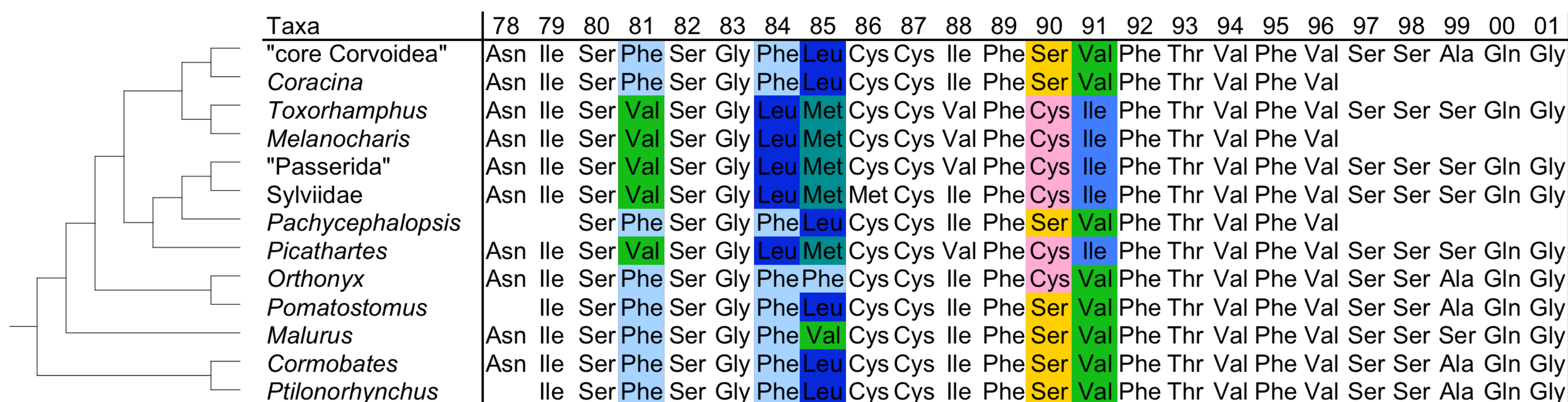
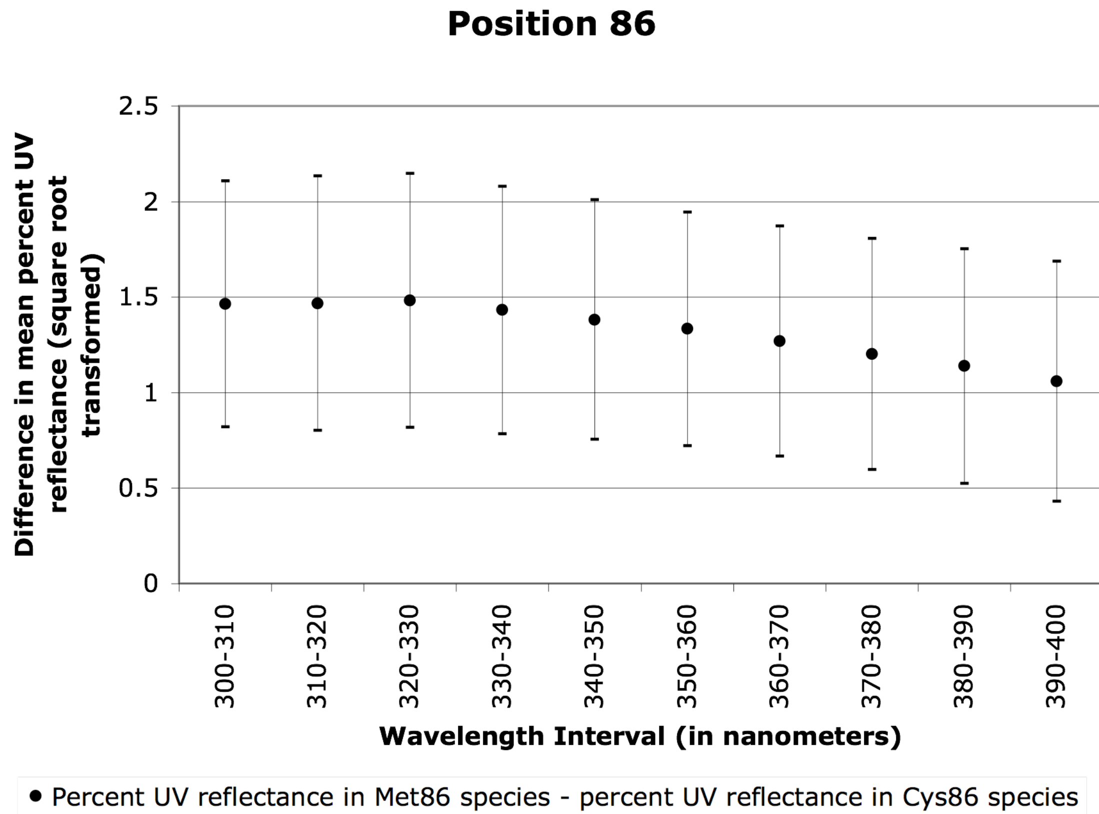


Figure 11



Taxa	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	00	01
"core Corvoidea"	Asn	Ile	Ser	Phe	Ser	Gly	Phe	Leu	Cys	Cys	Ile	Phe	Ser	Val	Phe	Thr	Val	Phe	Val	Ser	Ser	Ala	Gln	Gly
Coracina	Asn	Ile	Ser	Phe	Ser	Gly	Phe	Leu	Cys	Cys	Ile	Phe	Ser	Val	Phe	Thr	Val	Phe	Val					
Toxorhamphus	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Melanocharis	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val					
"Passerida"	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Sylviidae	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Met	Cys	Ile	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Pachycephalopsis			Ser	Phe	Ser	Gly	Phe	Leu	Cys	Cys	Ile	Phe	Ser	Val	Phe	Thr	Val	Phe	Val					
Picathartes	Asn	Ile	Ser	Val	Ser	Gly	Leu	Met	Cys	Cys	Val	Phe	Cys	Ile	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Orthonyx	Asn	Ile	Ser	Phe	Ser	Gly	Phe	Phe	Cys	Cys	Ile	Phe	Cys	Val	Phe	Thr	Val	Phe	Val	Ser	Ser	Ala	Gln	Gly
Pomatostomus		Ile	Ser	Phe	Ser	Gly	Phe	Leu	Cys	Cys	Ile	Phe	Ser	Val	Phe	Thr	Val	Phe	Val	Ser	Ser	Ala	Gln	Gly
Malurus	Asn	Ile	Ser	Phe	Ser	Gly	Phe	Val	Cys	Cys	Ile	Phe	Ser	Val	Phe	Thr	Val	Phe	Val	Ser	Ser	Ser	Gln	Gly
Cormobates	Asn	Ile	Ser	Phe	Ser	Gly	Phe	Leu	Cys	Cys	Ile	Phe	Ser	Val	Phe	Thr	Val	Phe	Val	Ser	Ser	Ala	Gln	Gly
Ptilonorhynchus		Ile	Ser	Phe	Ser	Gly	Phe	Leu	Cys	Cys	Ile	Phe	Ser	Val	Phe	Thr	Val	Phe	Val	Ser	Ser	Ala	Gln	Gly

Figure 12



APPENDICIES

Appendix A

List of all taxa included in the bowerbird phylogeny; museum specimen numbers included with number of individuals; AMNH- American Museum of Natural History, BM- The Natural History Museum

Table: Specimen list

Scientific name	Common name	# of individuals
Ingroup taxa		
Ailuroedus buccoides	white-eared catbird	1
Ai. crassirostris	green catbird	4
Ai. melanotis	spotted catbird	2
Amblyornis flavifrons	golden-fronted bowerbird	2 AMNH679121, BM1939.12.9.13
Am. inornatus	Vogelkop bowerbird	7
Am. macgregoriae	Macgregor's bowerbird	3
Am. papuensis	Archbold's bowerbird	3
Am. subalaris	streaked bowerbird	1
Chlamydera cerviniventris	fawn-breasted bowerbird	4
Ch. guttata	western bowerbird	4
Ch. lauterbachii	yellow-breasted bowerbird	1
Ch. maculata	spotted bowerbird	2
Ch. nuchalis	great bowerbird	2
Prionodura newtoniania	golden bowerbird	2
Ptilonorhynchus violaceus	satin bowerbird	3
Scenopoeetes dentirostris	tooth-billed bowerbird	2
Sericulus ardens	flame bowerbird	1 BM1916.5.30.1018
Se. aureus	masked bowerbird	1 BM99.11.3.3
Se. bakeri	Adelbert bowerbird	1 AMNH791268
Se. chrysocephalus	regent bowerbird	3
Outgroup taxa		
Cormobates leucophaeus	white-throated treecreeper	1
Malurus leucopterus	white-winged fairy-wren	1
Xanthotis flaviventer	tawny-breasted honeyeater	1

Appendix B

Primers used in polymerase chain reaction (PCR) and cycle-sequencing reactions for the bowerbird phylogeny; primer sequences may be slightly modified from the original referenced primer sequence

Table: Primer list

Locus	Primer name	Direction	Primer sequence (5' to 3')	Reference
Mitochondria				
Cytb				
	L14990	forward	CCATCCAACATCTCAGCATGATGAA	Kocher et al. 1989
	Cytb-1k	forward	TCCAACATCTCAGCATGATGAAA	Kocher et al. 1989
	Cytb-S2H	forward	GAATCTACTACGGCTCATAC	developed by R.C.F.
	Cyt-b2RC	forward	TGAGGACAAATATCCTTCTGAGG	developed by R.C.F.
	Cytb_wow-25F	forward	CCCATTAGGCATCCCATCAGA	developed by P.B.Z.
	Cytb_C-29F	forward	GCCAACCCACTCTCCACACCCA	developed by P.B.Z.
	Cytb-x	reverse	AGGTTTCGGATTAGTCAGCC	developed by R.C.F.
	Cytb-2k	reverse	TCAGAATGATATTTGTCCTCA	Kocher et al. 1989
	Cytb-wow	reverse	ATGGGTGGAATGGAATTTTGTC	developed by R.C.F.
	Cytb-c	reverse	AATAGGAAGTATCATTCGGGTTTG	Kocher et al. 1989
	H15916	reverse	ATGAAGGGATGTTCTACTGGTTG	Edwards et al. 1991
ND2				
	L5215	forward	TATCGGGCCCATACCCCGAAAAT	Helm-Bychowski & Cracraft 1983
	L5219	forward	CCCATACCCCGAAAATGATG	Sorenson et al. 1999
	Pt_L5329B	forward	CAAAATCTCACCACCCACGAGCC	developed by P.B.Z.
	L5421B	forward	AAGCCACAATCAAGTATTTCC	developed by P.B.Z.
	Pt_L5419	forward	GAAGCTGCAACAAAATACTT	developed by P.B.Z.
	Pt_L5473B	forward	TTCTCCAGCATATCCAACGC	developed by P.B.Z.
	L5758	forward	GGCTGAATRGGMCTNAAYCARAC	Sorenson et al. 1999
	Pt_L5758A	forward	GGATGAATGGGGYTAAAYCARAC	developed by P.B.Z.
	Pt_L5969	forward	AACTATCAACAYTAATAACCTCRTG	developed by P.B.Z.
	Pt_L6113	forward	AATGCCAGGCGTAGGTAGAA	developed by P.B.Z.
	Pt_H5419	reverse	AAGTATTTTGTTGCAGCTTCAAT	developed by P.B.Z.
	H5766	reverse	GGATGAGAAGGCTAGGATTTTKCG	Sorenson et al. 1999
	Pt_H5578A	reverse	CCTTGGAGTACTTCTGGGAATCARA	developed by P.B.Z.
	Pt_H5766A	reverse	GGATGAGAAGGCYARGATTTTKCG	developed by P.B.Z.
	Pt_H5977	reverse	GKCCKGCTAGAGAKAGTAGTGTGA	developed by P.B.Z.
	H6113	reverse	CAGTATGCAAGTCGGAGGTAGAAG	A. Baker pers. comm. To R.C.F.
	H6313	reverse	CTCTTATTTAAGGCTTTGAAGGC	Sorenson et al. 1999
Nuclear introns				

AK				
	AK5a+	forward	ATGCTGCGGGACGCCATGTTGG	Shapiro & Dumbacher 2001
	AK5b+	forward	ATTGACGGCTACCCTCGCGAGGTG	Shapiro & Dumbacher 2001
	AK1.5b+3	forward	AAGCAGGGAGAGGAGTTTGA	developed by P.B.Z.
	AK1f	forward	AAAAAGGTGAGGGCTGTGC	developed by P.B.Z.
	AK2f	forward	CATGTGGAGKGAGGCAGA	developed by P.B.Z.
	AK6c-	reverse	CACCCGCCCGCTGGTCTCTCC	Shapiro & Dumbacher 2001
	AK6c-2	reverse	GCTGGTCTCTCCCCGCTTC	developed by P.B.Z.
	AK6d-	reverse	GTTCGGTAGCCTTGTAGTACGTCTCC	Shapiro & Dumbacher 2001
	AK6e-	reverse	CCTTGTAGAAGGCGATGACGGGTTC	Shapiro & Dumbacher 2001
	AK1r	reverse	ACCATCGTGTCAGCACTGTT	developed by P.B.Z.
	AK2r	reverse	GAGGGGACAYGGTGGTCA	developed by P.B.Z.
Fib				
	Fib-BI7U	forward	GGAGAAAACAGGACAATGACAATTCAC	Primmer et al. 2002
	SerFib1f	forward	TGGATGGTATGTACCTGCACT	developed by P.B.Z.
	FIB1f	forward	TGGATGGTATGTACYTGCACT	developed by P.B.Z.
	FIB2f	forward	TGCTGTTCTCTTGGCATAGM	developed by P.B.Z.
	FIB3f	forward	TCAGAAGACAGGAGCTCAGTTG	developed by P.B.Z.
	FIB4f	forward	CCTTCTGAGTGTRCTCTGTAGC	developed by P.B.Z.
	Fib-BI7L	reverse	TCCCCAGTAGTATCTGCCATTA	Primmer et al. 2002
	FIB1r	reverse	GKCTATGCCAAGAGAACAGC	developed by P.B.Z.
	FIB2r	reverse	TTCCTACTCAGTGTCCTCAGCA	developed by P.B.Z.
	FIB3r	reverse	AACYACARTTACTTGCAGTTCA	developed by P.B.Z.
	FIB4r	reverse	TTGGATCTGCAGTTAACCTGAT	developed by P.B.Z.
Gapdh				
	Gapdh-F	forward	ACCTTTCATGCGGGTGCTGGCATTGC	Primmer et al. 2002
	Gapdh1f	forward	TGGCTCCAACTTGAAACAGTC	developed by P.B.Z.
	Gapdh2fa	forward	CAAGCTGGTTTCCTGGTAGG	developed by P.B.Z.
	Gapdh-R	reverse	CATCAAGTCCACAACACGGTTGCTGTA	Primmer et al. 2002
	Gapdh1r	reverse	CAGGGCTRACCCATTTCTTA	developed by P.B.Z.
	Gapdh2ra	reverse	TGTTTCAAGTTGGAGCCACTC	developed by P.B.Z.
ODC				
	ODC-F	forward	GACTCCAAAGCAGTTTGTCGTCTCAGTGT	Primmer et al. 2002
	ODC1f	forward	TCGTTGGAGTTAGGTGAGCTG	developed by P.B.Z.
	ODC2f	forward	TCGTTGGAATTTTTGAGGTC	developed by P.B.Z.
	ODC-R	reverse	TCTTCAGAGCCAGGGAAGCCACCACCAAT	Primmer et al. 2002
	ODC1r	reverse	GGGTCTGTACATCCACTTCCA	developed by P.B.Z.
	ODC2r	reverse	CAGCAACACTGTCAAKAAATCA	developed by P.B.Z.
RP40				
	RP40-F	forward	GGGCCTGATGTGGTGGATGCTGGC	Primmer et al. 2002
	RP401f	forward	AGCACCCATGGGAAGTCAT	developed by P.B.Z.
	RP402f	forward	CACTTGAATGTGGTGGTTGG	developed by P.B.Z.
	RP40-R	reverse	GCTTTCTCAGCAGCAGCCTGCTC	Primmer et al. 2002
	RP401r	reverse	ATTGGSAAAACCTGTCCTCA	developed by P.B.Z.

TGFβ2				
	TGFβ2-F	forward	GAAGCGTGCTCTAGATGCTG	Primmer et al. 2002
	TGFβ21f	forward	TTGTTTTAGGTAACTATGCCTCCA	developed by P.B.Z.
	TGFβ23f	forward	GTCCTCCAGGGAAGCCATR	developed by P.B.Z.
	TGFβ2-R	reverse	AGGCAGCAATTATCCTGCAC	Primmer et al. 2002
	TGFβ21r	reverse	CTGTGGGATTGGAGACCACT	developed by P.B.Z.
Trop				
	TROP-F	forward	AATGGCTGCAGAGGATAA	Primmer et al. 2002
	TROP-R	reverse	TCCTCTTCAAGCTCAGCACA	Primmer et al. 2002
Nuclear exon				
RAG-1				
	R13C	forward	TCTGAATGGAAATTCAAGCTCTT	K. Barker pers. comm.
	R15	forward	TCGCTAAGGTTTTCAAGATTGA	Groth & Barrowclough 1999
	R17	forward	CCCTCCTGCTGGTATCCTTGCTT	Groth & Barrowclough 1999
	R19	forward	GTCACTGGGAGGCAGATCTTCCA	Groth & Barrowclough 1999
	R21	forward	GGATCTTTGAGGAAGTAAAGCCCAA	Groth & Barrowclough 1999
	R23	forward	TACAAGAATCCTGATGTGTGTAA	Groth & Barrowclough 1999
	R16	reverse	GTTTGGGGAGTGGGGTTGCCA	Groth & Barrowclough 1999
	R18	reverse	GATGCTGCCTCGGTCGGCCACCTTT	Groth & Barrowclough 1999
	R20	reverse	CCATCTATAATTCCCACTTCTGT	Groth & Barrowclough 1999
	R22	reverse	GAATGTTCTCAGGATGCCTCCCAT	Groth & Barrowclough 1999
	R24	reverse	GCCTCTACTGTCTCTTTGGACAT	Groth & Barrowclough 1999
	R2I	reverse	GAGGTATATAGCCAGTGATGCTT	Barker et al. 2002
	RAG1-anc1L	forward	AAACACCTCAGGAAGAAGATGAA	developed by R.C.F.
	RAG1-anc2L	forward	AGGTTCCACTTACATTTGTACCC	developed by R.C.F.
	RAG1-anc3L	forward	GAGAAAGAAGAGGGCGGTGA	developed by R.C.F.
	RAG1-anc4L	forward	CTGTCTGGCCATCCGAATC	developed by R.C.F.
	RAG1-anc5L	forward	GGTTACCACCCGTTTGAGTG	developed by R.C.F.
	RAG1-anc6L	forward	GCTGAAAATCTGGAGCGATA	developed by R.C.F.
	RAG1-anc7L	forward	GCCTTAAAGGACATGGAGGA	developed by R.C.F.
	RAG1-anc10L	forward	TATCTGAAGATGAAGCCGGTGT	developed by R.C.F.
	RAG1-anc1R	reverse	CATGAGGATCGCCACACTG	developed by R.C.F.
	RAG1-	reverse	TGGGTTGGACCTCCATATTT	developed by R.C.F.

anc2R			
RAG1-anc3R	reverse	TATGATACTGACTACAGCTGAGAAA	developed by R.C.F.
RAG1-anc4R	reverse	ACATTTTTCAGGGGAGGTTTC	developed by R.C.F.
RAG1-anc5R	reverse	GCCTTCCAAGATCTCCTCCT	developed by R.C.F.
RAG1-anc6R	reverse	CTTCTTCCTGAGGTGTTTGTCA	developed by R.C.F.
RAG1-anc7R	reverse	TGTGAAAGAAAAGCGAACAGC	developed by R.C.F.
RAG1-anc10R	reverse	TTTCGATGATTTCAGGAACATGAG	developed by R.C.F.
MRAG102f	forward	TGSCATGAAAACACAAGACAA	developed by P.B.Z.
MRAG109f	forward	ACAAACAAGCAGATGAATTGGA	developed by P.B.Z.
MRAG119f	forward	ACTTCCACAAAACGCTTGCT	developed by P.B.Z.
MRAG102r	reverse	CTCGCACATCAATCTTGAACA	developed by P.B.Z.
MRAG109r	reverse	CTCGAATGGGTGGTAACCTG	developed by P.B.Z.
MRAG119r	reverse	AGCCAGTGATGCTTCAAGAC	developed by P.B.Z.

Appendix C

Details of polymerase chain reaction (PCR) recipes and programs used to amplify gene regions used in to estimate the bowerbird phylogeny

Most PCR programs followed this program: 94° 10', (92° 45", X° 1', 72° 1') 35 cycles, 72° 5' where X is the annealing temperature reported in the table. Those reactions marked with M were used to amplify samples taken from museum specimens and used this program: 94° 10', (92° 45", X° 1', 72° 1') 45 cycles, 72° 5'. Ranges are given when different conditions were needed to amplify from all taxa. All primers were used both for the amplification and cycle-sequencing steps. Additional RAG1 primers were used to in the cycle sequencing reactions.

Table: Primer sets with reported $MgCl_2$ concentration and annealing temperatures; M- museum PCR program, KB- follows K. Barker's (pers. communication) PCR program (see also Chesser and ten Have 2007)

Locus	Primer set	$MgCl_2$ concentration (mM)	Annealing temperature (°C)	PCR program
Mitochondria				
Cytb	L14990/Cytb-X	2.0	52	M
	L14990/Cytb-wow	1.5	52-55	
	L14990/Cytb-c	1.5	58	
	L14990/H15916	1.5	52-55	
	1k/2k	1.5	60	
	1k/Cytb-wow	1.5	58	
	1k/Cytb-c	1.5	58	
	S2H/2k	2.0	54	M
	2RC/Cytb-wow	2.0	52	M
	2RC/H15916	1.5	52-55	
	Cytb_wow-29F/Cytb-c	2.0	52	M
	Cytb_C-25F/H15916	2.0	52	M
ND2	L5215/Pt_H5419	2.0	54	M
	L5215/H5766	1.5	58-61	

	L5215/Pt_H5766A	1.5	56-58	
	L5215/H6113	2.0	54-60	
	L5219/Pt_H5419	2.0	54	M
	L5219/H5766	1.5	58-61	
	L5219/Pt_H5766A	1.5	58	
	L5219/H6113	1.5	55-60	
	Pt_L5329/Pt_H5578A	1.5-2.0	53-58	M
	Pt_L5419/Pt_H5578A	1.5-2.0	53-58	M
	L5421B/Pt_H5578A	1.5-2.0	53-58	M
	Pt_L5473B/Pt_H5766A	1.5-2.0	53-58	M
	Pt_L5473/Pt_H5977	1.5	58	
	L5758/H6313	1.5	58-61	
	Pt_L5758A/Pt_H5977	1.5-2.0	53-58	M
	Pt_L5758A/H6113	1.5	58	
	Pt_L5758A/H6313	1.5	53-57	
	Pt_L5969/H6113	1.5-2.0	53-58	M
	Pt_L5969/H6313	1.5	53-58	
	Pt_L6113/H6313	1.5-2.0	53-58	M
Nuclear introns				
AK	5a+/6c-	1.5	59-61	
	5a+/6d-	1.5	54-56	
	5a+/6e-	1.5	54-56	
	5b+/6c-	1.5-2.0	56-61	
	5b+3/6c-2	2.0	56-58	
	AK1f/AK1r	2.0	55	M
	AK2f/AK2r	2.0	50-53	M
Fib	Fib-BI7U/Fib-BI7L	1.5	60	
	Fib1f/Fib1r	2.0	50-53	M
	Fib2f/Fib2r	2.0	50-53	M
	Fib3f/Fib3r	2.0	50-53	M
	Fib4f/Fib4r	2.0	50-53	M
	SerFib1f/Fib-BI7L	2.0	52	M
Gapdh	Gapdh-F/Gapdh-R	1.5	60	
	Gapdh1f/Gapdh1r	2.0	50-53	M
	Gapdh2fa/Gapdh2ra	2.0	50-53	M
ODC	ODC-F/ODC-R	1.5	60	
	ODC1f/ODC1r	2.0	52-54	M
	ODC2f/ODC2r	2.0	50-53	M
RP40	RP40-F/RP40-R	1.5	60	
	RP401f/RP401r	2.0	50-53	M
	RP402f/RP40-R	2.0	50-53	M
TGFβ2	TGFβ2-F/TGFβ2-R	1.5	60	
	TGFβ21f/TGFβ21r	2.0	50-53	M
	TGFβ23f/TGFβ2-R	2.0	50-53	M
Trop	Trop-F/Trop-R	1.5	60	
Nuclear exon				

RAG1	R13C/R18	1.5	KB	
	R17/R22	1.5	KB	
	R21/R2I	1.5	KB	
	RAG1-anc1R/RAG1-anc1L	2.0	50	M
	RAG1-anc2R/RAG1-anc2L	2.0	50	M
	RAG1-anc3R/RAG1-anc3L	2.0	50	M
	RAG1-anc4R/RAG1-anc4L	2.0	50	M
	RAG1-anc5R/RAG1-anc5L	2.0	50	M
	RAG1-anc6R/RAG1-anc6L	2.0	50	M
	RAG1-anc7R/RAG1-anc7L	2.0	50	M
	RAG1-anc10R/RAG1-anc10L	2.0	50	M
	MRAG102f/MRAG102r	2.0	50	M
	MRAG109f/MRAG109r	2.0	50	M
	MRAG119f/MRAG119r	2.0	50	M

Appendix D

Alternative partitioning scheme analysis for the bowerbird phylogenetic dataset

Bayes factors, calculated from harmonic log likelihood scores, were compared to test whether alternative partitionings of the 9001 bp dataset resulted in better tree estimations. Comparisons were made in relation to the most partitioned dataset (P31). Differences in log likelihood scores are likely due to the inclusion of additional model parameters, as the topology estimates among analyses remain the same.

Table: Analysis of alternative partitionings with associated harmonic log likelihood scores, calculated Bayes factors, and the estimated topology

Partitioning scheme	Total partitions	Mitoch-ondrial partitions	Nuclear partitions	log likelihood	Bayes Factor	2*BF	topology
No partitions	0	combined	combined	-34903.28	2411.32	4822.64	A(CB,M)
Comparing nuclear partitions							
P1	10	Cytb; ND2 & tRNAs	each region	-33994.61	1502.65	3005.30	A(CB,M)
P2	12	Cytb; ND2 & tRNAs	each region; RAG1 codon positions	-33361.51	869.55	1739.10	A(CB,M)
P3	10	Cytb; ND2 & tRNAs	each intron; all exons combined	-34077.43	1585.47	3170.94	A(CB,M)
P4	12	Cytb; ND2 & tRNAs	each intron; all exon codon positions combined	-33371.49	879.53	1759.06	A(CB,M)
P7	13	Cytb; ND2 & tRNAs	each intron; RAG1 codons; remaining exons combined	-33199.77	707.81	1415.62	A(CB,M)
Comparing mitochondrial partitions							
P1	10	Cytb; ND2 & tRNAs	each region	-33994.61	1502.65	3005.30	A(CB,M)
P10	13	Cytb; ND2; tRNAs	each region; RAG1 codon positions	-33401.96	910.00	1820.00	A(CB,M)
P18	14	Cytb & ND2 codons combined; tRNAs	each region; RAG1 codon positions	-32844.11	352.15	704.30	A(CB,M)
P26	17	Cytb codons; ND2 codons; tRNAs	each region; RAG1 codon positions	-32508.32	16.36	32.72	A(CB,M)
Final partition (P7 and P26 combined)							
P31	18	Cytb codons; ND2 codons; tRNAs	each intron; RAG1 codons; remaining exons combined	-32491.96			A(CB,M)

Appendix E

Analysis of alternative bowerbird topologies

Four methods were used to test for significance of alternative topologies in relation to the most likely topology based on the 9001 bp, non-partitioned, dataset. The approximately unbiased test and Shimodaira-Hasegawa test were performed in CONSEL v0.1j (Shimodaira and Hasegawa 2001) and PAUP* v4.0b10 (Swofford 2004), respectively. Analyses of Bayes factors (BF; table 1), calculated from harmonic log likelihood scores of alternative topologies, suggests a change in opinion for the more likely topology, in this case A(CB,M). Additionally, analysis of insertions and deletions (indels; table 2) suggest three indels support A(CB,M) and no indels support CB(A,M).

Table 1: Analysis of Bayes factors

Topology	Harmonic Log likelihood	Bayes factor	2*BF
CB(A,M)	-34910.37		
A(CB,M)	-34903.11	7.26	14.52

Table 2: Analysis of indels describing gene region where in indel is found and length of indel. Suggested topology is that estimated when assuming species with similar indel size are most closely related; category 1 indels suggest placement of the catbirds, 2 suggests placement of the ingroup and outgroup taxa, 3 suggest relationships among the outgroups, 4 is within catbirds, 5 is within *Chlamydera*, 6 is within *Amblyornis*, 7 is within the avenue bower-builders, 8 represents unexpected relationships; O- outgroups,

A- avenue bower-builders, M- maypole bower-builders, CB- catbirds, Cor- *Cormobates leucophaeus*, Mal- *Malurus leucopterus*, Xan- *Xanthotis flaviventer*, Ind- individual, RT- remaining taxa

Gene	Start	Length	Suggested topology
Gapdh	122	2	1: O(A(M,CB))
Gapdh	287	5	1: O(A(M,CB))
Trop	152	1	1: O(A(M,CB))
AK	91	1	2: O(A,M,CB)
AK	207	13	2: O(A,M,CB)
Fib	420	1	2: O(A,M,CB)
Fib	536	7	2: O(A,M,CB)
ODC	563	1	2: O(A,M,CB)
ODC	591	10	2: O(A,M,CB)
RAG1	1031	3	2: O(A,M,CB)
RP40	126	2	2: O(A,M,CB)
TGFβ2	141	1	2: O(A,M,CB)
Fib	532	4	3: Cor,Mal(RT)
RP40	125	1	3: Cor,Mal(RT)
TGFβ2	142	1	3: Cor,Mal(RT)
AK	114	31	3: Mal,Xan(RT)
AK	555	15	3: Mal,Xan(RT)
Gapdh	184	1	3: Mal,Xan(RT)
Gapdh	234	8	3: Mal,Xan(RT)
Gapdh	296	1	3: Mal,Xan(RT)
ODC	543	2	3: Mal,Xan(RT)
TGFβ2	209	6	3: Mal,Xan(RT)
Fib	311	5	4: *Ai.crassirostris*,*Ai.melanotis*(RT)
ODC	409	2	4: *Ai.crassirostris*,*Ai.melanotis*(RT)
RP40	112	1	4: *Ai.crassirostris*,*Ai.melanotis*(RT)
AK	466	5	4: *Ai.crassirostris*,*Ai.buccoides*(RT)
AK	403	61	5: *Ch.nuchalis*,*Ch.maculata*[Ind#1](*Ch.maculata*[Ind#2],RT)
Fib	902	18	5: *Ch.lauterbachii*,*Ch.cerviniventris*(RT)
ODC	188	24	6: O,A,M(*Am.papuensis*,*Am.macgregoriae*)
ODC	501	1	7: O,CB,M,*Ch.guttata*,*Ch.nuchalis*,*Se.aureus*,*Se.ardens*,*S.bakeri* (*Ch.cerviniventris*,*Ch.maculata*,*Ch.lauterbachii*,*Se.chrysocephalus*, *Pt.violaceus*)
AK	104	41	8: Cor,*Sericulus*(RT)
Fib	922	1	8: Cor,Xan,*Ai.crassirostris*,*Ai.buccoides*(RT)
Fib	931	1	8: Xan,*Ai.buccoides*(RT)
Fib	901	1	8: *Ch.lauterbachii*,*Ch.cerviniventris*,Cor(RT)
RP40	123	1	8: Xan,Mal,A,CB,*Sc.dentirostris*(Cor,M minus *Sc.dentirostris*)
TGFβ2	581	4	8: Mal,Xan,M,CB(Cor,A)

Appendix F

List of taxa used in the *Sericulus* phylogeny

Table: Specimen identification and location of field collected samples; number next to specimen represents location on figure 4; AMNH- American Museum of Natural History, NHM- The Natural History Museum, NSW- New South Wales, NG- New Guinea, AUST- Australia

***Species* (number for reference on Fig. 4)**	GenBank Accession numbers **EU341380-EU341493**	
Sericulus ardens		
(1) Museum: AMNH Accession #: 427633 Location: Tarara, NG	(2) Museum: NHM Accession #: 1916.5.30.1018 Location: Waitakwa River, NG	
Sericulus aureus		
(3) Museum: NHM Accession #: 99.11.3.3 Location: Mt. Moari, Arfak Mts., NG	(4) Museum: AMNH Accession #: 342290 Location: Bernhard Camp, NG	(5) Museum: AMNH Accession #: 303007 Location: Weyland Mts., NG
Sericulus bakeri		
(6) Museum: AMNH Accession #: 791276 Location: Nawawu, Adelbert Mts., NG	(7) Museum: AMNH Accession #: 791268 Location: Memenga, Adelbert Mts., NG	
Sericulus chrysocephalus		
(8) Museum: AMNH Accession #: 703207 Location: Bunya Mts., Queensland, AUST	(9) Location: Wallaby Creek, NSW, AUST	

Appendix G

Primers used to amplify and sequence gene regions for use in estimation of the *Sericulus* phylogeny

Table: Primer list

Gene	Primer pair	Primers	Sequence	Reference
Cytb	S2H/2k	Cytb2S2H	GAATCTACTACGGCTCATAC	Developed by RCF
	2RC/wow	Cytb2.RC	TGAGGACAAATATCCTTCTGAGG	Developed by RCF
		Cytb2.wow	ATGGGTGGAATGGAATTTTGTC	Developed by RCF
	wow-29/C	Cytb2.wow-29	CCCATTAGGCATCCCATCAGA	This study
		Cytb2.C	AATAGGAAGTATCATTCGGGTTTG	Modified from Kocher et al. 1989
	CorL/Cor3	CorL	ACTGCGACAAAATCCCATTC	Developed by RCF
		Cor3	GACTCCTCCTAGTTTATTTGGG	Developed by RCF
ND2	L5219/H5419	Pt_L5219	CCCATACCCCGAAAATGAGWSG	Modified from Sorenson et al. 1999
		Pt_H5419	AAGTATTTTGTTGCAGCTTCAAT	This study
	L5419/H5578	Pt_L5419	GAAGCTGCAACAAAATACTT	This study
		Pt_H5578a	CCTTGGAGTACTTCTGGGAATCARA	This study
	L5758/H5977	Pt_L5758	GGATGAATRGGVYTMAAYCARAC	Modified from Sorenson et al. 1999
		Pt_H5977a	GKCCKGCTAGAGAKAGWAGTGTGA	This study
	L5969/H6113	Pt_L5969	AACTATCAACAYTAATAACCTCRTG	This study
		H6113	CAGTATGCAAGTCGGAGGTAGAAG	Developed by A. Baker, per. comm.
Fib	Fib1f/r	Fib1f	TGGATGGTATGTACYTGCACT	This study
		Fib1r	GKCTATGCCAAGAGAACAGC	This study
	Fib2f/r	Fib2f	TGCTGTTCTCTTGGCATAGM	This study
		Fib2r	TTCCTACTCAGTGTCCTCAGCA	This study
	Fib4f/r	Fib4f	CCTTCTGAGTGTRCTCTGTAGC	This study
		Fib4r	TTGGATCTGCAGTTAACCTGAT	This study
Gapdh	Gapdh1f/r	Gapdh1f	TGGCTCCAACTTGAAACAGTC	This study
		Gapdh1r	CAGGGCTRACCCATTTCTTA	This study
ODC	ODC1f/r	ODC1f	TCGTTGGAGTTAGGTGAGCTG	This study
		ODC1r	GGGTCTGTACATCCACTTCCA	This study
	ODC2f/r	ODC2f	TCGTTGGAATTTTTGAGGTC	This study
		ODC2r	CAGCAACACTGTCAAKAAATCA	This study
RP40	RP401f/r	RP401f	AGCACCCATGGGAAGTCAT	This study
		RP401r	ATTGGSAAAACCTGTCCTCA	This study
	RP402f/RP40r	RP402f	CACTTGAATGTGGTGGTTGG	This study
TGFB2	TGFB21f/r	TGFB21f	TTGTTTTAGGTAACTATGCCTCCA	This study
		TGFB21r	CTGTGGGATTGGAGACCACT	This study
	TGFB23f/r	TGFB23f	AGTGGTCTCCAATCCCACAG	This study
		TGFB23r	AGCTYATGGCTTCCCTGGAG	This study
RAG-1	MRAG09f/r	MRAG09f	ACAAACAAGCAGATGAATTGGA	This study
		MRAG09r	CAGGTTACCACCCATTCGAG	This study
	MRAG19f/r	MRAG19f	ACTTCCACAAAACGCTTGCT	This study
		MRAG19r	GTCTTGAAGCATCACTGGCT	This study
	RAG1-anc2L/R	RAG1-anc2L	AGGTTCCACTTACATTTGTACCC	Developed by RCF
		RAG1-anc2R	AAATATGGAGGTCCAACCCA	Developed by RCF
	RAG1-anc7L/R	RAG1-anc7L	GCCTTAAAGGACATGGAGGA	Developed by RCF
		RAG1-and7R	GCTGTTCGCTTTTCTTTCACA	Developed by RCF

Appendix H

List of primers used to amplify and sequence the bowerbird short-wavelength-sensitive 1 (SWS1) opsin gene

Table: Primer list and length of gene region once aligned

Primer name	Direction	Primer sequence (5' to 3')	Length of aligned region in bps
SWS1Af1	forward	TGGACGAGGAAGAGTTTTACC	922
SWS1Ar1	reverse	CAGGAAGGCCAAGGACCA	
SWS1Bf1	forward	CCAGGGATACTTYGTCTTCG	1233
SWS1Bf2	forward	CGCTCAACTACATCCTGGTG	1308
SWS1Br1	reverse	GGGACGATGAAGCAGAAGAT	
SWS1Df1	forward	GAAGGCAGAGCGGGAGGT	813
SWS1Dr3	reverse	GACCTGGCTGGAGGACACAGAG	

Appendix I

List of passeriform species from which UV reflectance and/or short-wavelength-sensitive 1 (SWS1) opsin gene sequences were collected

Table: Sampled species where ** represents the same species used by Barker et al. (2004) and * represents the same genus; included are museum specimen number for species scanned for UV reflectance (N – U. S. National Museum (Smithsonian Institution), A – American Museum of Natural History), and GenBank accession numbers for previously reported short-wavelength-sensitive 1 (SWS1) nucleotide sequences; references for GenBank submitted sequences: 1- Ödeen & Hästad 2003, 2- Browne et al. GenBank direct submission 2006, 3- Odeen et al. 2009, 4- Yokoyama et al. 2000, 5- Das et al. 1999, 6- Raman & Andersson GenBank direct submission 2003, 7- Okano et al. 1992

Family	Species	Museum specimen #	GenBank ACCN #
Acanthisittidae	*Acanthisitta chloris**		
Alaudidae	*Alauda arvensis***	N325284	
Cardinalidae	*Cardinalis cardinalis***		
Campephagidae	*Coracina caeruleogrisea**	N264029	
	*Lalage lewcomela***	N405957	
Climacteridae	*Cormobates leucophaeus***	N278196	
Corvidae	*Corvus corone***	N424598	AY227176[1]
	Corvus frugilegus		DQ451006[2]
	*Corvus monedula**	N424606	AY227177[1]
	*Cyanocitta cristata***		
Cracticidae	*Gymnorhina tibicen***	N278089	
Dendrocolaptidae	*Xiphorhynchus guttatus*	N327378	
Dicruridae	*Dicrurus modestus**	N460407	
Emberizidae	*Melospiza melodia*		
	Zonotrichia albicollis		
Estrildidae	*Amadina fasciata*	N212013	FJ440639[3]
	Chloebia gouldiae	N575731	FJ440640[3]
	Lonchura maja	N180954	FJ440641[3]
	Lonchura punctulata		

	Neochmia modesta		FJ440642[3]
	Neochmia temporalis		
	Taeniopygia guttata	N324942	AF222331[4]
Eurylaimidae	*Psarisomus dalhousiae***	N408283	
	*Smithornis rufolateralis***	N462709	
Furnariidae	*Xenops minutus*	N371740	
Fringillidae	*Carpodicus mexicanus*		
	Serinus canaris	N213311	AJ277922[5]
Icteridae	*Dolichonyx oryzivorus*		FJ440643[3]
	Molothrus ater		
Irenidae	*Chloropsis aurifrons**	N459444	
	*Irena cyanogaster***		
Laniidae	*Lanius excubitor***	N102930	
Maluridae	*Malurus leucopterus**	N335216	
Melanocharitidae	*Melanocharis nigra***	N584734	
	*Toxorhamphus novaeguineae***	N585043	
Meliphagidae	*Meliphaga analoga***	N519048	
	*Meliphaga lewinii**	N278121	
Mimidae	*Dumetella carolinensis*	N357008 N469049	
	*Mimus polyglottos**	N531557 N599327	
	Oreoscoptes montanus	N141589 N258834	
Monarchidae	*Monarcha chrysomela***	N265021	
Motacillidae	*Motacilla alba**	N107007	
	*Motacilla lugens**	N201494	
Muscicapidae	*Luscinia calliope*	N450942	AY274226[6]
	Luscinia svecica	N77777	AY274225[6]
Nectariniidae	*Nectarinia aspasia**	N572079	
	*Nectarinia chloropygia**	N460480	
	*Nectarinia olivacea***	N517338	
	Nectarinia modesta	N278997	
Oriolidae	*Oriolus brachyrhynchus**	N462717	
Orthonychidae	*Orthonyx spaldingii***	N279335	
Pachycephalidae	*Colluricincla boweri**	N279192	
	*Colluricincla megarhyncha**	N609219	
	*Pachycephala simplex**	N406047	
	*Pitohui ferrugineus**	N509031	
Paradisaeidae	*Cicinnurus magnificus*	N145527	
	Epimachus fastuosus	N116896	
	Lophorina superba	N125204	
	*Paradisaea raggiana***	N172226	
	Parotia lawesii	N189644	
	*Ptiloris magnificus***	N126013	
Paradoxornithidae	*Paradoxornis webbianus*	N303297	
Paridae	*Parus atricapillus**		
	*Parus caeruleus**	N113810	AY274220[6]
	*Parus major***	N208596	

	*Parus palustris**		AY274222[6]
Passeridae	*Passer domesticus**	N379071	
	*Passer montanus***		
Petroicidae	*Tregellasia capito**	N335073	
Phasianidae	*Gallus gallus***		NM205438[7]
Picathartidae	*Picathartes gymnocephalus***	N526311	
Pipridae	*Manacus manacus*	N627103	AY227182[1]
	Manacus vitellinus		
	*Pipra mentalis**	N525602	
Pittidae	*Pitta versicolor**	N189647	
Ploceidae	*Euplectes afer*	N518777	AY274223[5]
	Euplectes orix	N517686	AY274224[5]
	*Ploceus nigricollis**	N253878	
Polioptilidae	*Polioptila dumicola**	N284034	
Pomatostomidae	*Pomatostomus isidorei***	N518975	
Ptilonorhynchidae	*Ailuroedus buccoides**	A421010 A679748 A679749 A809352	
	*Ailuroedus crassirostris**	N278103	
	*Ailuroedus melanotis***		
	Amblyornis inornatus		
	Amblyornis macgregoriae	N584871 N584872	
	Amblyornis subalaris		
	Amblyornis papoensis		
	Chlamydera cerviniventris		
	Chlamydera guttata	A679153 A810989	
	Chlamydera lauterbachii		
	Chlamydera maculata		
	Chlamydera nuchalis		
	Prionodura newtoniania	A679447 A679496 A679497 A679506 N279343 N279344	
	*Ptilonorhynchus violaceus***	*minor*: A679421 N15104 N279345 *violaceus*: N278096 N278100	
	Scenopoeetes dentirostris	N121199 N131198	
	Sericulus chrysocephalus	A679320 A679332 N121226 N189646	

Pycnonotidae	*Pycnonotus cafer**		
	*Pycnonotus latirostris**	N520370	
	*Pycnonotus melanicterus**		
Rhipiduridae	*Rhipidura aureola**		
	*Rhipidura rufiventris**	N377687	
Sittidae	*Sitta carolinensis***	N229988	
	*Sitta castanea**		
Sturnidae	*Acridotheres tristis*	N525814	
	Aplonis metallica	N571827	
	Lamprotornis superbus	N247655	
	*Sturnus vulgaris***	N529714	AY227180[1]
Sylviidae	*Phylloscopus inornatus*		
	Phylloscopus reguloides		
	Phylloscopus trivirgatus		
	Phylloscopus trochilus	N546943	AY227181[1]
Thamnophilidae	*Cercomacra tyrannina*	N402343	
Timaliidae	*Garrulax erythrocephalus**		
	*Garrulax leucolophus**	N536143	
	*Garrulax monilegar**	N450618	
	*Garrulax nuchalis**		
	*Garrulax pectoralis**		
	Leiothrix argentauris		
	Leiothrix lutea	N585932	
	Yuhina nigrimenta		
	Yuhina bakeri		
Troglodytidae	*Thryothorus ludovicianus*		
	*Troglodytes aedon***	N417471	
Turdidae	*Hylocichla mustelina*		
	Turdus merula	N325285	FJ440637[3]
	*Turdus migratorius**		
Tyrannidae	*Tityra semifasciata***	N189146	
	Myiarchus tyrannulus	N369528	AY227183[1]
	*Tyrannus tyrannus***	N422434	
Vireonidae	*Vireo griseus**	N364060	
Zosteropidae	*Zosterops erythropleurus**		
	*Zosterops senegalensis***	N570030	
	*Zosterops japonicus**	N114731	
	*Zosterops montana**		
	*Zosterops palpebrosus**		

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