The Ecology of Blue-crowned Manakins (*Lepidothrix coronata*)

A Comparison Study of Biometric Sexing Using Discriminant Analyses

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August 2013

A thesis submitted to the Postgraduate School College of Medical, Veterinary and Life Sciences in fulfilment of the requirements for a Masters of Science by Research Ecology, University of Glasgow

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Abstract

Blue-crowned manakins (*Lepidothrix coronata)* exhibit neotenic retention of subadult monomorphic plumage in sexually mature males. Definitively plumaged adult *L. coronata* are dichromatic, with males displaying a black body and blue crown while females retain green colouration characteristic of Pipridae species. Male neoteny and the reliance on soft tissue colouration to identify females make mature monomorphic *L. coronata* indistinguishable in the field, presenting research and management difficulties. The application of biometric measurements with discriminant function analysis (DFA) offers a practical methodology to sex *L. coronata.* Three DFA methods were compared using *L. coronata* of definitive plumage and known sex to determine the best modelling methodology for future applications. A linear discriminant analysis was performed using biometric measurements and combined with a principal component analyses. Quadratic discriminant analysis was performed using biometric measurements as a comparison to linear methodologies. Linear and quadratic discriminant analyses of biometric measurements produce a 92.86 and 91.2 per cent accuracy sexing definitively plumaged *L. coronata,* indicating applicability of statistical modelling as a potential solution for future field applications.

Acknowledgements

I would like to acknowledge my advisor Dr Stewart White, who has patiently answered my questions, provided sound advice, and introduced me to research in San José de Payamino. I would like to express my appreciation to Dr Richard Preziosi of the University of Manchester for being amazingly considerate and helpful. I would like to thank the University of Glasgow 2012 Ecuador Expedition Crew for their assistance in the collection of my data. I would especially like to offer my appreciation for the numerous groups and individuals who contributed data to the Payamino Project, without whom this research would not have been possible. I am particularly grateful for the assistance and support of the Timburi Cocha Scientific Research Station and the community of San José de Payamino, who continue to support and invest in scientific research. I would like to offer my special thanks to the US-UK Fulbright Commission for endorsing my studies at the University of Glasgow and for facilitating one of the best years of my life.

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Section 1: Introduction

Sexual selection was presented by Charles Darwin in *The Descent of Man, and Selection in Relation to Sex* (1871) as an explanation for the existence of secondary sexual characteristics. Secondary sexual characteristics are adaptations that increase intrasexual competitive advantage but may not aid negotiation of the environment or increase the likelihood of survival (Molles Jr. 2009; Campbell et al. 1999; Clutton-Brock 2007; Andersson 1994a). Sexual selection mechanisms act to maximise breeding potential by increasing access to mates or by increasing mate attraction. Favoured secondary sexual characteristics and behaviours are continued in offspring and contribute to the gene pool as aspects of fitness.

Ecological pressures for sexual selection increase with gender limitations on the energetic investment in reproduction and the intensity of intrasexual competition for breeding (Owens & Thompson 1994; Clutton-Brock 2007; Andersson 1994a). Female selection increases male variation due to breeding competition for the limited number of fertile females (Owens & Thompson 1994; Clutton-Brock 2007; Andersson & Iwasa 1996). Male selection occurs where variations in female reproduction are increased and there are fitness advantages in mate selection (Clutton-Brock 2007; Clutton-Brock 2009).

Mechanisms of sexual selection include pre-copulation competition, fitness advertisement, and post-copulation competition (Andersson & Iwasa 1996; Clutton-Brock 2007; Andersson 1994a; Johnson & Burley 1998). In avian species pre-copulation sexual selection mechanisms include fitness advertisement through plumage colouration, ornamental feathers, song, building infrastructure, and sexual size dimorphism (Clutton-Brock 2007; Owens & Hartley 1998; Owens & Thompson 1994; Andersson & Iwasa 1996). Post-copulation selection mechanisms include sperm competition and female sperm selection

(Dean et al. 2011; Briskie & Montgomerie 1993; Lifjeld et al. 1994; Møller & Ninni 1998; Andersson & Iwasa 1996). Avian species may present multiple secondary sexual characteristics applicable to particular aspects of intrasexual competition or mate attraction (Møller & Pomiankowski 1993; Pryke et al. 2001).

Plumage colouration in avian species is an indication of mate quality in both sexes, where bright colouration, elaborate patterns, or ornamentation signal mate fitness (Hill 1993; Stein & Uy 2006; Doucet 2002; Gomez et al. 2013). The brightness of feather colouration is an indicator of offspring fitness, the number of offspring, and the ability to provide paternal care in socially monogamous species (Siefferman & Hill 2005; Balenger et al. 2009; Siefferman & Hill 2003; Møller & Birkhead 1994). In bluebirds (*Sialia* spp.), the brightness of feathers is an indication of foraging abilities. Pigmentation formed during moult is affected by the quantity and quality of food, with consistent feeding reflected in brighter plumage (Siefferman & Hill 2005; Siefferman & Hill 2003; Balenger et al. 2009).

Contrast in plumage patterns is hypothesized by Hasson (1991) to be a mechanism to accentuate feather contour and wear by allowing the edges of feathers to be distinctive. Feather glossiness and pattern can emphasise contour and low wear, indicating foraging capabilities and overall quality of feather structure (Hasson 1991; Fitzpatrick 1998). Plumage brightness in male passerines can also reflect immunocompetence and resistance to endoparasites and viruses (Hamilton & Zuk 1982; Hamilton & Poulin 1997; Pruett-Jones et al. 1990; Lindstrom & Lundstrom 2000).

Plumage ornamentation in males and females acts as an advertisement of genetic quality, mate fitness, and capacity for parental investment (Møller 1993; Amundsen 2000; Saino et al. 1997; Winquist & Lemon 1994). Ornamental plumage is most common in males, where ornaments may also be utilised in intrasexual competition to assert dominance (Pryke

et al. 2001; Andersson & Andersson 1994). In species with long retrice feathers, an extensive tail is a highly visible indication of male fitness to competitive males and potential mates (Pryke et al. 2001; Møller & Pomiankowski 1993).

Male tail length is positively correlated with the number of fathered offspring in social and extra-pairings. Ornamental retrice feathers of the male barn swallow (*Hirundo rustica)* have also been linked to an increased female reproductive input (Møller 1991; de Lope & Møller 1993). Adaptation of long tail plumage by males can act to accentuate mobility to females when combined with display (Byers et al. 2010). Ornament condition, especially feather length, displays resistance to parasites and has been correlated to lower infection of feather mites and endoparasites (Hoglund et al. 1992; Höglund & Sheldon 1998; Møller 1990).

The duration, frequency, and complexity of songs are a mechanism of intrasexual competition and intersexual mate attraction in some avian species. Song and auditory displays signify genetic quality and mate endurance is associated with high song frequency or complexity (Searcy 1992; Searcy & Andersson 1986; Catchpole 1987). The complexity and duration of songs in the European starling (*Sturnus vulgaris)*, the common whitethroat (*Sylvia communis)*, and aquatic warbler (*Acrocephalus paludicola)* are correlated to increased female selection (Eens et al. 1991; Catchpole & Leisler 1996; Balsby 2000). The frequency and complexity of responses to competitor song serve as a method of claiming dominance, securing territories, and increasing mate access in intrasexual competition (Searcy & Yasukawa 1990). The ability to produce frequent, complex songs is also an indication of healthiness and resistance to parasitic infections (Redpath et al. 2000; Catchpole & Leisler 1996; Hamilton & Zuk 1982; Gilman et al. 2007).

Avian species such as the ruffed grouse (*Bonasa umbellus)*, Calypte genus of hummingbirds and the club-winged manakin (*Machaeropterus deliciosus)* also utilise sonation or mechanical noise in intrasexual competition and intersexual selection (Feo & Clark 2010; Bostwick & Prum 2003; Prum 1998; Clark 2008; Samuel et al. 1974; Clark & Feo 2010). Sonation produced by moving wing or tail feathers is a common component of Neotropical species mate attraction (Feo & Clark 2010; Bostwick & Prum 2003; Bostwick 2000; Prum 1998; Clark 2008). Mechanical noise such as drumming by downy woodpeckers (*Picoides pubescens)* and wing popping in the Pipra genus of Pipridae also serve as a method of asserting dominance in intrasexual competition (Bostwick 2000; Prum 1998; Kilham 1974a; Kilham 1974b).

The construction of nests or elaborate bowers is a courtship mechanism employed by male bowerbirds (Ptilonorhynchidae) and weavers (Ploceidae) (Quader 2006; Borgia 1985). Male building acts as an ornament for the purpose of female selection, where the quality of nest construction determines reproductive investment and hatchling success (Quader 2006; Borgia 1985; Quader 2003). The nest height and placement by male weaverbirds is correlated to paternal investment and hatchling survival (Quader 2006; Quader 2003). Intrasexual competition includes sabotage of nests and bowers and fighting for space in desired building locations (Borgia 1985).

Elaborate courtship displays, leks, are a mechanism of mate attraction in the ruffed grouse, cotingas (Cotingidae) and Pipridae species that is often coupled with physical adornments and sound mechanisms (Durães 2009; Endler & Thery 1996; Anciães & Prum 2008; Prum 1998; Feo & Clark 2010; Clark 2008). Courtship displays include a physical repertoire of movements, such as dances, bobs, and flights combined with auditory display (DuVal 2007; Rosselli et al. 2002; Andrew 1961; Payne 1984). Male displays act to emphasise secondary sexual characteristics such as plumage colouration, lengthy retrice

feathers, or mechanical sound in fitness advertisement (Durães 2009; Endler & Thery 1996; Anciães & Prum 2008; Prum 1998; Payne 1984).

Dominance competition has aided in the adaptation of sexual size dimorphism where one gender exhibits larger biometrics than the other (Owens & Hartley 1998; Webster 1997; Cabana et al. 1982; Chardine & Morris 1989; Searcy & Yasukawa 1981). Increased biometric ratios between sexes occurs in species with multiple pair copulations and with increased energetic investment differences in paternal care (Owens & Hartley 1998; Andersson & Iwasa 1996; Clutton-Brock 2007). Male biased sexual size dimorphism is commonly observed in mass or wing chord. Female biased or reverse sexual size dimorphism is observed in birds of prey (Falconiformes), seabirds such as skuas (Stercorariidae), and some passerines such as species of Pipridae (Payne 1984; Widén 1984; Catry et al. 1999; Phillips et al. 2002; Lundberg 1986). Sexual size dimorphism can act concurrently with secondary sexual characteristics or other mechanisms of sexual selection, increasing opportunities for mate selection, attraction, and territory security (Owens & Hartley 1998; Webster 1997; Cabana et al. 1982; Chardine & Morris 1989; Searcy & Yasukawa 1981).

The most common form of sexual size dimorphism is exhibited in males as a larger mass or wing chord due to intrasexual competition for female selection (Owens & Hartley 1998; Webster 1997; Cabana et al. 2013; Chardine & Morris 2012; Searcy & Yasukawa 1981). Intrasexual competition for territories can produce an increased body size, allowing individuals breeding dominance, earlier first clutches, improved access to resources, increased mate choices, and preferred nest sites (Haggerty 2006; Searcy & Yasukawa 1981; Webster 1997; Langston et al. 1990). Correspondingly, intrasexual competition can also produce adaptive benefits for a smaller body size in males and females when an increase in mobility is important for individual survival and courtships displays (Payne 1984; Hasson 1991; Phillips et al. 2002).

Studies of phylogeny and evolutionary biology necessitate an understanding of social gender roles, characteristics of reproductive success, and the population change driven by sexual selection. The mechanisms of sexual selection aid in shaping a species' evolutionary history by changing the propensity of a particular phenotype in a population and, when coupled with ecological circumstance, can produce speciation (Molles Jr. 2009; Campbell et al. 1999; Andersson 1994b; Clutton-Brock 2007). Individual fitness is an expression of genetic phenotype and reproductive success over time changes the gene pool of a population, causing shifts in social behaviour and morphological adaptation (Molles Jr. 2009; 1999; Prum 1994; Prum 1998; Winkler 2000; Milá et al. 2009).

Numerous avian species can be sexed using hands-on techniques developed by ornithologists and banders. Captured individuals can be sexed by assessing plumage colouration or pattern, colouration of soft tissue, wing chord length, and seasonally by brood patch or cloacal protuberance (Proctor & Lynch 1993; Balmer et al. 2008). In dichromatic species, colouration, pattern and plumage ornamentation indicates a male comparative to a cryptic or less vibrant female plumage (Hasson 1991; Clutton-Brock 2009; Siefferman & Hill 2005; Balenger et al. 2009; Siefferman & Hill 2003; Møller; Møller & Birkhead 1994). In cryptic or monomorphic species, plumage colouration, patterning and ornamentation are not viable sexing methodologies.

Alternative methods to plumage based sexing include examination of other physical features. The colouration of soft tissue, typically the iris, is used as an indicator of age and sex in some Falconiformes and Passeriformes (Mueller et al. 1976; Snyder & Snyder 1974; Kirwan & Green 2011; Balmer et al. 2008). In breeding season, brood patches and cloaca protuberance can be used to identify males and females in many species. The exposed skin of brood patches can be used to identify females, except in species where males assume broodiness or share in egg incubation (Bailey 1952; Proctor & Lynch 1993). With the

exception of species that do not have a cloaca, such as waterfowl, an engorged cloaca protuberance is characteristic of a male (Boersma & Davies 1987; Salt 1954; Swanson & Rappole 1992; Wolfson 1952).

Behavioural observation can be used to identify gender in species with conspicuous social or parental roles. Species with gender specific nest building or with courtship displays can be sexed by exhibited behaviours (Hoglund et al. 1990; Payne 1984; Quader 2006). As with brood patches and cloacal proturberance, behavioural sexing is limited by seasonality and is dependent on parental and mate attraction roles by sex. Alternative sexing methodologies such as surgical examination of gonads, molecular analysis, and statistical analysis are utilised when secondary sexual characteristics or gender identifying behaviour cannot be used. Surgical, molecular or statistical analyses facilitate sex identification of monomorphic species independent of expressed gender characteristics.

Laparotomy and laparoscopy surgical procedures allow the sex organs to be viewed in live specimens. Laparotomy can be utilised in both field and laboratory studies to observe the gonads by making an incision between the last two ribs on the left side to provide a view of the ovary or testicle (Bailey 1953; Lawson & Kittle 1971; Griffiths 2000). Laparoscopy uses the same surgical incision, but employs an endoscope to maneuvre inside the body cavity (Richner 1989; Bush et al. 1978). The use of an endoscope in laparoscopy reduces risk of organ puncture and provides better views of the gonads (Richner 1989; Bush et al. 1978). Alternatively, cloacascopy is a laparoscopic procedure where the endoscope placed into the cloacal vent to sex large avian species by physiological characteristics of the cloaca (Sladen 1978; Gancz & Taylor; Wagner, 1995; Ritzman, 2008).

Mortality as a result of surgical sexing procedures is as low as one per cent and is primarily due to risk of puncturing air sacs or organs and negative anaesthetic reactions

(Bailey, 1953; Lawson & Kittle, 1971; Richner, 1989). The morality associated with infection is minimal and procedure recovery may leave individuals more vulnerable to predation (Richner, 1989). The expense of surgical sexing techniques and the restrictions of invasive procedures by scientific permit can make it impractical for many studies.

Molecular sexing methods include cytological sex identification, DNA hybridization, and polymerase chain reaction (PCR). Molecular analyses utilise the CHD gene, which contains the W and Z sex chromosomes (Griffiths 2000; Dubiec & Zagalska-Neubauer 2006; Ellegren 2000). Gender is identified by female heterogamety and male homogametic Z chromosomes (Griffiths 2000; Griffiths et al. 1998; Dubiec & Zagalska-Neubauer 2006; Ellegren 2000). The CHD gene can be isolated from blood, feather, or tissue samples for gender identification (Hogan et al. 2008; Rudnick et al. 2005; Dubiec & Zagalska-Neubauer 2006).

Cytological sex identification uses cultured cell nuclei and the morphology of the sex chromosomes to determine gender (Griffiths 2000; Shields 1982; Rutkowska & Badyaev 2008). Cell cultures are treated with bleach at the metaphase stage of mitosis to create chromosome spreads that are prepared with stain to highlight chromosome morphology for light microscopy (Griffiths 2000; Dubiec & Zagalska-Neubauer 2006; Rutkowska & Badyaev 2008). Chromosome spreads utilise the distinguishable difference in size between the avian sex chromosomes to make a gender determination, where the larger Z macrochromosome are distinct comparative to the smaller W microchromosome (Griffiths 2000; Dubiec & Zagalska-Neubauer 2006; Rutkowska & Badyaev 2008). Cytological molecular sexing is uncommon compared to other molecular methodologies due to the difficulty of producing adequate cell cultures from biological samples other than feather pulp, which may limit testing to times of moult (Griffiths 2000).

DNA hybridization creates bands of DNA sequences that can be used to explore a genome (Griffiths 2000; Dubiec & Zagalska-Neubauer 2006). DNA hybridization for the purpose of sexing focuses on the identification of female specific W chromosome characteristics (Griffiths 2000; Dubiec & Zagalska-Neubauer 2006). The W chromosome carries a small amount of unique coding DNA, which is fragmented with an enzyme that targets specific nucleotide sequences and separated with electrophoresis from the non-coding sequences (Griffiths 2000; Griffiths et al. 1996; Dubiec & Zagalska-Neubauer 2006). The DNA fragments are transferred to a filter membrane using Southern blotting and a probe is hybridized to DNA sequences to mark areas of interest (Griffiths 2000; Griffiths et al. 1996; Dubiec & Zagalska-Neubauer 2006). The resulting range of sequence bands are assessed to determine if they are female specific (Griffiths et al. 1996; Griffiths 2000; Dubiec & Zagalska-Neubauer 2006). DNA hybridization is used less frequently than PCR methods due to the length of the Southern blot process.

PCR testing amplifies DNA fragments through the use of two primer sequences and a sample of DNA (Griffiths et al. 1996; Griffiths 2000). The primer sequences complement the DNA sample and facilitate a controlled hybridization that copies the fragment of interest multiple times. PCR amplifies either the RAPD or AFLP sequences for inspection. The AFLP test limited by the inability to amplify the same genetic sequence in different avian species (Griffiths 2000). The RAPD test amplifies the CHD1-W gene, a functional gene that can be used equitably for avian sexing with the exception of ratites (Griffiths et al. 1996; Griffiths 2000).

Biometric sexing exploits sexual size dimorphism of morphological characteristics to sex individuals by statistical classification analyses such as logistic regression or discriminant function analysis (DFA) (Crawley 2013). Statistical classification analyses determine if a set

of variables, such as biometric measurements, can be used successfully to predict group membership to a gender category (Claude 2008; Crawley 2013; Everitt & Hothorn 2011; Henderson & Seaby 2008). Common biometric variables include wing chord, weight, bill, total head, tail, tarsus, and toe lengths but additional biometric measurements may be used and vary dependent on species (Santiago-Alarcon & Parker 2007; Bluso et al. 2006; Kavanagh 1988).

Logistic regression analysis is employed with a predicted classification restricted to two group categories (Everitt & Hothorn 2011; Claude 2008; Crawley 2013). Logistic regression predicts group membership by creating an equation that best calculates maximum probability of classifying the observed data to group category. Classification is determined based on the probability of group membership assuming a continuous relationship between the dependent and independent variables (Everitt & Hothorn 2006; Claude 2008; Crawley 2013). Logistic regression applied to avian sexing can produce high sexing accuracies, as exemplified by Fuertes et al. (2010) and Rodriguez, Pugesek and Diem (1996) who studied water rails (*Rallus aquaticus*) with 80% accuracy and California gulls (*Larus californicus*) with 99.2% and 97.0% sex classification accuracies.

Discriminant function analysis (DFA) is the most common statistical classification technique used for biometric sexing of monomorphic species such as seabirds, shorebirds, and cryptic passerines (Desrochers 1990; Puebla-Olivares & Figueroa-Esquivel 2009; Arizaga et al. 2008; Ryder & Durães 2005; Bluso et al. 2006). DFA determines group membership by using the centroid of the independent variables associated with each of the dependent group categories (Henderson & Seaby 2008; Everitt & Hothorn 2011; Crawley 2013). The centroids are used to assign a variable coefficient for the discriminating equation, which is then used to assign individuals a discriminant score and categorise them to a group

(Henderson & Seaby 2008; Everitt & Hothorn 2011). As observed in scientific literature, DFA sexing can produce a 80% to 90% accuracy in classifying individuals to a sex category (Arizaga et al. 2008; Bluso et al. 2006; Ryder & Durães 2005; Puebla-Olivares & Figueroa-Esquivel 2009).

Pipridae are a family of frugivorous neotropical passerines that exhibit extended cryptic plumage and neoteny where mature males maintain a juvenile or female appearance (Doucet et al. 2007; Duval 2005; McDonald 1989; Foster 1987; Kirwan & Green 2011). Pipridae species have dichromatic definitive plumage. Females are an olivaceous shade of green and males commonly develop a black body with bright colours on the head, rump, wings, or legs (Kirwan & Green 2011; Heindl 2002; Duval 2005; Payne 1984). In some species, males develop ornamental retrice feathers or retain green body plumage in combination with bright coloured ornamental plumage (Kirwan & Green 2011; Ridgely & Greenfield 2001; Heindl 2002; Duval 2005). The development of male definitive dichromatic plumage occurs after three years in most species, but can occur as late as five years after a series of predefinative moults (Doucet et al. 2007; Duval 2005; McDonald 1989; Foster 1987; Kirwan & Green 2011; Ryder & Durães 2005). Males reach sexual maturity prior to the development of definitive plumage and the retention of predefinative moult is a neotenical characteristic (Doucet et al. 2007; Duval 2005; McDonald 1989; Foster 1987; Kirwan & Green 2011).

Pipridae undergo a partial predefinative moult, which enables juveniles to be distinguished from subadults by contrast in the greater covert feathers and redness of the iris (Ryder & Durães 2005; Doucet et al. 2007; Duval 2005; Kirwan & Green 2011). The second predefinative moult occurs approximately a year after the first partial moult. During the second predefinative moult, males may begin to show aspects of adult definitive plumage

(Ryder & Durães 2005; Doucet et al. 2007; Duval 2005; Kirwan & Green 2011). Males achieve definitive adult plumage during the third predefinative moult, while females will occasionally produce bright crown feathers (Graves et al. 1983; Kirwan & Green 2011). Males that do not exhibit identifying characteristics after the second predefinative moult or those that only acquire a few crown feathers can be confused with mature females (Ryder & Durães 2005; Doucet et al. 2007; Duval 2005). In these instances, gender is indistinguishable.

Pipridae neotenic plumage is hypothesized to be an adaptation to lek courtship displays. Pipridae species employ both cooperative and exploded leks where the alpha male copulates with females almost exclusively (Durães, et al., 2009; Kirwan & Green, 2011; McDonald, 1989; McDonald, 1993). The neotenic delay of definitive plumage is hypothesized as a strategy to gain access to mates, resources, reduce male-male aggression between young and alpha males, or to acquire courtship display skills (Foster 1987; McDonald 1993). Neotenous plumage allows young males who will not copulate a spot in the "queue" where they can eventually become a beta or alpha male and increase their ability to successfully complete for copulation (McDonald 1993).

The blue-crowned manakin (*Lepidothrix coronata)* is a Pipridae superspecies constituting nine subspecies ranging from southern Costa Rica to northern Bolivia (Kirwan & Green 2011). *L. coronata* reaches sexual maturity at two years, with the development of adult definitive plumage at three years (Kirwan & Green 2011; Ryder & Durães 2005). Male definitive plumage consists of a black body with a bright blue crown. Females retain monomorphic green plumage with occasional blue head feathers and a vibrant red iris (Doucet, et al., 2007; Ridgely & Greenfield, 2001). As with other Pipridae species, male *L. coronata* have a neotenous plumage that make them indistinguishable from monomorphic females (Ridgely & Greenfield 2001; Ryder & Durães 2005; Kirwan & Green 2011).

The inability to sex mature monomorphic *L. coronata* presents practical problems for management and research. Without a sexing methodology, populations cannot be accurately estimated. Evolutionary history and evaluations of population fitness cannot be adequately accounted for due to the lack of knowledge about intrasexual competition and adaptive benefits of neoteny in male-male interactions. Assessments of the effect of ecological pressures on a species by gender, such as intersexual resource competition, are similarly limited by the inability to identify sex. Establishing a sexing methodology will facilitate greater understanding of the role of natural and sexual selection in *L. coronata* adaptations and aid in management, conservation, and scientific research.

Molecular sexing and biometric sexing methods have both been successfully applied to sex Pipridae species (Doucet et al. 2007; Duval 2005; Mendenhall et al. 2010; Ryder & Durães 2005). Currently, Ryder and Durães (2005) have published the only study to use molecular sexing on *L. coronata* as a means to determine sex of individuals post second predefinative moult. Mendenhall et al. (2010) and Ryder and Durães (2005) employed DFA analysis as a sexing method with respective 92.8% and 93.6 % classification accuracies for other species of Pipridae. Currently, DFA and other biometric sexing methods have not been applied to *L. coronata* in scientific literature and remain untested.

The following research evaluates the ability of discriminant function analysis to accurately classify the San José de Payamino, Ecuador population of *L. coronata* to the correct gender group. The known adult *L. coronata* sampled were assessed to determine if the population exhibited reverse sexual size dimorphism, which is common of other small bodied Pipridae (Ryder & Durães 2005; Mendenhall et al. 2010; Kirwan & Green 2011; Payne 1984; Théry 1997). The hypothesis that adult male *L. coronata* have significantly smaller biometric measurements than females was evaluated using a MANOVA and a paired t-test. A sexual

size dimorphism index was calculated by dividing the mean value for males by the mean value for females for each biometric measurement to indicate differences in body size (Gill & Vonhof 2006). The biometric measurements of definitively plumaged *L. coronata* were assessed for naturally occurring gender divisions that can be utilised to determine sex in the field by exploring the data with a recursive partitioning tree model.

The method of using statistical models and biometric measurements to sex *L. coronata* was selected due to limitations presented by field conditions and restrictive legislation on biological sampling from Decision 391: Common Regime on Access to Genetic Resources by the Comunidad de Andina (The Commission of the Cartagena Agreement 1992). DFA is the most frequent technique used to sex avian species and discriminant models also allows for an established model to predict the group membership of novel data (Pohar et al. 2004; Everitt & Hothorn 2011; Claude 2008). The discriminant models evaluated as part of this research will be applicable for use in further study to sex newly collected individuals.

Three discriminant models were compared to determine the best model fit for *L. coronata*. Two variants of a linear discriminant models were utilised, with one version using the results of a principal component analysis (PCA) as independent variable inputs rather than the biometrics measured. A combined analysis of principal components and a linear discrimination was used to couple the pattern extraction capabilities of the PCA to refine grouping criteria used in the DFA (Jombart et al. 2010; Darroch & Mosimann 1985; Zhu 2006). A quadratic discriminant model was used as an alternative to a linear model due to minor data abnormalities, as a linear discriminant model is sensitive to homogeneity and outliers (Lachenbruch et al. 1973; Nakanishi & Sato 1985; Pohar et al. 2004).

Section 2: Materials and Methods

2.1 Study Site

The study was conducted at San José de Payamino, Ecuador at the Timburi Cocha Scientific Research Station. San José de Payamino is a small rural Kichwa village in the Orellena Province, located on the low-lying slopes of the northern range of the Andes Mountains inside Sumaco National Park. San José de Payamino features both primary and secondary Amazonian rainforest and varzea forest. As part of an active agricultural Kichwa community, the research station is located in the middle of maintained secondary and tertiary forest. The entirety of this study was conducted in secondary forest, due to the overall abundance of the habitat around the Timburi Cocha Scientific Research Station.

2.2 Sampling Method

2.2.1 Mist Net Sampling

The majority of the 2012 and 2013 field seasons used two 18 meter long and 2.75 meter high mist nets with 32mm mesh. In prior field seasons or when there was adequate aid in ringing, as many as six mist nets were used at one time. Mist net sites were initially selected due to their use in past field seasons by Dr White during bioassay surveys. Mist net sites that had a high capture rate history for *L. coronata* were reused in both the 2012 and 2013 field seasons. Reutilisation of mist net sites due to capture rates for *L. coronata* were not applicable for field seasons prior to 2012, where *L. coronata* data was collected as a general bioassay.

Mist nets were erected in sites the day prior to use and furled until the following morning to be opened just after dawn. The nets were run from approximately 6 until 10:30 every

morning. After this time, avian activity naturally began to decline and the increase in heat became oppressive to avian movement. Mist net sampling was conducted every day for the duration of the field season unless prohibited by storm conditions.

During the course of the fieldwork, it became clear through observation that *L. coronata* is responsive to lure calls. *L. coronata* of male definitive plumage and indistinguishable monomorphic plumage were observed responding to recorded calls played by increasing the frequency of song in response and through the approach of birds to the player. Recordings of *L. coronata* were subsequently played using Phillips GoGear SA2MXX USB MP3 players and Panavox 60HZ-20KHZ portable speakers hidden beneath fallen leaves at mist nets.

The use of audio lures is associated with sex bias in capture, with an increased number of captured males comparative to females (Lecoq & Catry 2003). Audio lures are also associated with an overall increase in the number of captured individuals of both genders, allowing for a greater amount of biometrics sampling in a limited field period. The previous 12 years of *L. coronata* biometrics sampling without the use of audio lures was female biased (n=42) and an increase in the number of sampled definitive males (n=19) as a result of audio lure capture bias was deemed a positive addition to the collected data. Accordingly, the numbers of definitive male, not-adult male (subadult) and juvenile *L. coronata* caught increased with the use of recorded song.

The nets were checked every half hour for captured birds. Birds were placed into cloth bird bags after their removal from the mist net until they could be processed. Upon the completion of the sampling period, the mist nets were then shifted to the next site where they were erected and furled for the following morning. Exceptions were made for closures of nets due to heavy rain or high capture rates where the nets would be used in the same location twice in succession.

Captured *L. coronata* were identified as one of four field sex and age categories used by Dr White. Adult birds of discernable sex were categorised as male or female based on sexually dimorphic plumage, with females separated from monomorphic immatures by the redness of the iris and the occasional blue head feather (Doucet, et al., 2007; Ridgely & Greenfield, 2001). Immature *L. coronata* were categorised as juvenile due to contrast in wing converts and dull red irises or as not adult male if contrast was not present. Not adult male was the comprehensive category used for subadult and mature *L. coronata* that cannot be positively aged or sexed due to monomorphic plumage (i.e. non-male plumaged).

2.2.2 Biometric Measurements

Five biometric measurements were part of the processing procedure in the Payamino Project avian bioassays. Total head, bill, weight, wing, and tarsus measurements were taken according to the field standards put forward by the British Trust for Ornithology Ringers Manual (Balmer et al. 2008). Calipers were utilised to measure bill, total head, and tarsus lengths to the nearest 0.01 millimeter. The wing chord was measured with a one hundred millimeter wing rule to the nearest 0.1 millimeter. Weight was taken with either a 10-gram spring scale or electronic balance and was measured to the 0.1 gram.

Bill length was measured from the edge of the feathering at the start of the bill to the tip. The total head distance was measured from the back of the skull to the tip of the bill. Tarsus length was measured from the lower end of the knee joint to where the tarsus bone ends in the ankle, or just before the bend of the foot. Wing chord was measured from wing joint to the tip of longest primary feather (Balmer et al. 2008). Birds were weighed in cones constructed from a light and open plastic sheeting on the spring scale. The weight of the cone was subtracted to give the birds weight in grams. Alternatively, birds were placed into a plastic

tub, which was tared on the electronic balance before the bird was placed inside head first for weighing.

2.3 Analysis Methodology

2.3.1 Software

All data collected on *L. coronata* was stored in Microsoft Excel, which was also used to produce tables and spread sheets. Excel sheets were saved as comma separated values (csv) files and imported into R using the read.csv function for analyses (Crawley 2013; Beckerman & Petchey 2012). All statistical analyses were conducted in R (Team 2013) using a variety of statistical packages written for R to address various aspects of statistics. The platform RStudio was used in conjunction with the default R software console (RStudio 2013).

2.3.2 Data Preparation

Upon importation into the R software, the biometrics data was examined for errors using built in statistical functions and the moments package. The data was examined for outliers and tested for normality using QQ-plots, the Fligner-Killeen test of homogeneity of variances, the Shapiro-Wilk test of normality, D'Agnostino test for skewness, and a skewness parameter (Komsta & Novomestky 2012; Komsta 2013; Crawley 2013). Outliers were determined using the interquartile range, box and whisker plots, and histograms. Substantial outliers were removed from the data set due to the sensitivity of linear discriminant analysis to the presence of outliers; whereas, borderline outliers were kept to preserve the range of biometric measurements.

A MANOVA analysis was used to determine the potential variance in biometric measurements between field seasons. Variance between field seasons was taken into consideration due to the consistent change of novice student ringers within the Payamino Project with each season. Pseudoreplication from recaptured individuals was removed by averaging the biometric measurements for that individual within a data subset. Individuals who were captured first as immatures and recaptured as adults (n=3) were not averaged between age classifications, but they were averaged if captured multiple times as the same field category.

2.3.3 Sexual Size Dimorphism Calculation

A MANOVA analysis using the Pillai Criterion was conducted to determine the significance of the physiological difference between the sexes and potential of sexing with statistical models (Team 2013; Crawley 2013). The degree of *L. coronata* size dimorphism was determined by calculating the ratio of mean male and female biometric measures. An index of body size was created from the ratio of total head, bill, weight, wing chord, and tarsus measurements. The index was used to indicate the degree of sexual size dimorphism in the biometric measurements (Haggerty 2006; Webster 1997). The significance of the size difference between genders in each biometric variable was assessed in a paired t-test. The resulting P values were used to determine the variation within an individual physical characteristic.

2.3.4 Tree Analysis

A classification tree was constructed using a rpart package function further refined with a tree package function (B. Ripley 2013; Therneau et al. 2013; Team 2013). The combination of the tree and rpart packages produced the best fit for the *L. coronata* data. Tree analysis employs recursive partitioning, which groups data by similarities in response variables while maintaining the maximum distinction between variables (Strobl et al. 2009; Speybroeck 2009). Tree analysis was employed to explore the natural divisions within the biometric variables by sex in *L. coronata.* The graphical divisions in biometrics data was used as a reference when determining the importance of individual biometric variables in determining the sex group classification of *L. coronata* in the DFA models.

The rpart function differs from the tree function due to its built in ANOVA, which determines the division at each node and keeps the resulting trees simplified (Therneau et al. 2013; Terry et al. 2013). The tree function provides greater detail including the interactions that occur within the same variable within a sex category (Ripley & Ripley 2013; B. Ripley 2013). The classification tree of the *L. coronata* data was created allowing for all possible leaves. The model was systematically reduced using the cross-validated error associated with the size of the tree and reduced using the prune.tree function in the tree package (Ripley & Ripley 2013; B. Ripley 2013).

2.3.5 Discriminant Function Analysis

The discriminant function analyses were conducted with the MASS and klaR packages in R (M. B. Ripley 2013; Venables & Ripley 2002; Weihs et al. 2005). Two linear discriminant analyses (LDA) were used, comparing the accuracy of models classifying individuals by biometric measurements and principal components. The principal components were calculated in the default R package stats and used identically to the collected biometric variables in the model construction. Principal component analysis (PCA) was conducted to transform biometrics data into correlational interactions that represented data patterns and variable relationships. PCA is often coupled with LDA to increase the ability to extract patterns and to reduce the data input into models while maintaining variation. A single quadratic discriminant analysis (QDA) was conducted using the collected biometric measurements as input variables.

Pilot models were created with all biometric variables or principal components and fitted backward stepwise by removing variables with the highest calculated error rate. Error rates for the classification abilities of particular variables were assessed using a partition plot from the klaR package. Variables that produced the lowest error rates and the discriminant coefficients with the least weight were removed stepwise until the model was simplified to the most accurate classification.

The models were constructed using definitive plumaged and sexed *L. coronata* with a non-cross-validated and cross-validated equivalent. The models were constructed backward stepwise using the biometrics of definitively plumaged and sexed *L. coronata* and accessed based on classification accuracies with and without cross-validation. The use of backward stepwise construction was used to evaluate the efficiency and importance of individual biometric measurements (i.e. tarsus, wing) in the DFA models and to eliminate error from

model over-fitting. Leave one-out cross-validation was used to access the stability of model predictive abilities. Cross-validated models are unable to make predictions for novel data, necessitating the two equivalent analyses of the same model.

The DFA model group classifications were used to produce a comparison table to indicate the error and accuracy rates of the different discriminant methods. The resulting classifications of mature *L. coronata* were compared to the known sexes of individuals to determine the accuracy of classification based on biometric or principal component modelling. A comparison of model performance was utilised to determine a sexing method for future research.

Section 3: Results

3.1 Sexual Size Dimorphism

A total of 71 individual definitively plumaged *L. coronata* total head, bill, weight, wing chord, and tarsus records were collected over 13 years of sampling in San José de Payamino. Dr Stewart White conducted bioassays for the Payamino Project during the summer months as part of a student funded research expedition from the University of Glasgow or a university field course. A MANOVA test determined a negligible difference in biometric measurements taken between field seasons (Pillai Criterion= 0.62, F=1.2, df=13, 65, P>0.05) despite a turnover of inexperienced ringers participating in the project.

The MANOVA analysis conducted on the sexual variation between biometric measurements indicates sufficient cause for discriminant analysis (Pillai Criterion=0.64, F=21.8, df=1, 5, P<0.001). The MANOVA results demonstrate that head (F=67.9, P<0.001, s^2=0.41), weight (F=76.3, P<0.001, s^2=0.38), and wing chord (F=24.8, P<0.001, s^2=1.78) measurements had significant variation between the sexes. The calculated index of sexual size dimorphism and paired t-test evaluation clarify that female *L. coronata* are larger bodied than males (Table 3.1.1). The results of the MANOVA and sexual size dimorphism index provide sufficient evidence to accept the hypothesis of reverse sexual size dimorphism in the San José de Payamino population. The dimorphism of the head, weight, and wing chord biometric measurements provide enough variation to procede with discriminant sexing. Tarsus and bill measurements were determined to have insignificant variation.

Sexual Size Dimorphism Calculation						
	Male (n=27)		Female (n=44)			
	Mean	Max, Min	Mean	Max, Min	Dimorphism	P
Head	25.12 ± 0.42	25.9, 24.4	26.39 ± 0.74	28.5, 25.0	0.95	<0.0005
Bill	9.64 ± 0.93	11.9,8.4	9.89 ± 0.99	11.1, 7.7	0.97	0.2055
Weight	8.52 ± 0.53	9.5, 7.5	9.84 ± 0.65	11.5, 8.5	0.87	<0.0005
Wing	60.45 ± 1.23	63, 58	58.76 ± 1.54	62, 55	1.03	<0.0005
Tarsus	14.15 ± 1.2	16.8, 12	14.16 ± 1.2	16.8, 12	1	0.9821

Table 3.1.1: Calculated adult sexual size dimorphism index ratio by biometric variable

3.2 Classification Tree Analysis

The combined rpart and tree function classification provided the best fit and the tree with the most practical field utility. The simplification of the tree model using cross-validation error oversimplified the model to only two nodes, so the non-simplified model was utilised to provide greater detail. The first node depicts the division between mature male and female *L. coronata* by weight, which separates males as less than 8.96 grams from heavier females (Figure 3.2.1). Further detail is given in a range of male tarsus lengths.

Males that were heavier than 8.96 grams were separated from females by head and wing chord measurements, reaffirming the natural divisions in biometric measurements calculated in the previous section. Adult males have smaller head lengths than females and were separated by head lengths less than 25.85mm (Figure 3.2.1). Male *L. coronata* were further separated in the terminal node by having a larger wing chord, with measurements greater than 59.5 mm (Figure 3.2.1).

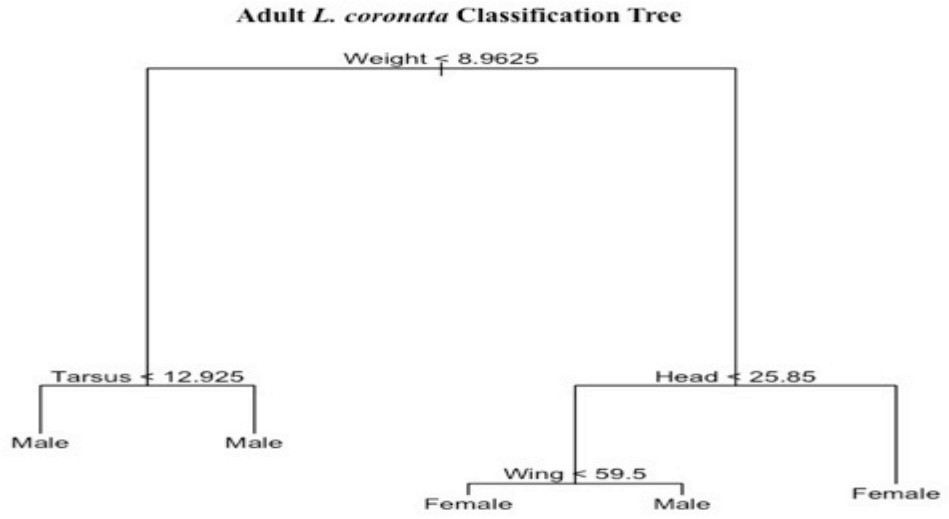

Figure 3.2.1: Graphical representation of sex differences in mature *L. coronata*

3.3 Discriminant Function Analysis Comparison

A total of 3 individuals, 2 females and 1 male, were excluded from the analyses due to missing weight data. The remaining 68 records were used in the DFA model comparison. The biometric LDA model was best fit backward stepwise with head, weight, and wing chord. These input variables were selected due to the least relative error in the partition plots and the weight of the linear coefficient values in the discriminant equation (Appendix C). The LDA/PCA model was constructed from the principal component analysis included in Appendix D. As with the biometrics LDA, the relative error and the values of the linear coefficients were used to select the first three principal components for the best fit. The QDA model backward stepwise simplification was best fit with head, weight, and wing biometric measurements due to low relative error rates of the pilot model (Appendix E).

The LDA model utilising biometric measurement inputs classified a total of 92.86 per cent of adult *L. coronata* accurately, misclassifying 3 males and 2 females (Table 3.3.1). The ability of the LDA model to accurately classify adult *L. coronata* was assessed through the leave one out cross-validated equivalent of the model. The cross-validation model had a classification accuracy of 89.71 and an additional male misclassification, a negligible difference that indicates stability in the model classification abilities (Table 3.3.1).

The LDA/PCA model classified a total of 89.06 per cent of adult *L. coronata* accurately with an increase in female misclassification from the biometrics LDA model (Table 3.3.1). Model cross-validation had an 88.23 per cent classification accuracy, indicative of the replicability of the classification results. Both LDA models were also used to produce a histogram of discriminant scores, graphically representing the ability of the function to separate the gender categories (Figure 3.3.1).

Discriminant Function Analysis Results						
	Non-Cross-Validated			Cross-Validated		
		Misclassified			Misclassified	
	Accuracy	Male (n=26)	Female (n=42)	Accuracy	Male (n=26)	Female (n=42)
LDA	92.86	2	3	89.71	3	4
LDA/PCA	89.06	2	5	88.23	3	5
QDA	91.2	3	3	89.71	3	4

Table 3.3.1 Comparison of discriminant analyses for accuracy and stability

The discriminant scores calculated by the biometrics LDA function in Figure (A) and the scores calculated by the LDA/PCA model in Figure (B) depict females in the top histogram and males in the bottom histogram (Figure 3.3.1). Male *L. coronata* in both models were assigned predominately positive discriminant scores while group female *L. coronata* were assigned negative discriminant scores. Both LDA models have a clear division between sex categories reflected in the distribution of the assigned discriminant scores, with a wider range of assigned scores present in the LDA/PCA model (Figure 3.3.1).

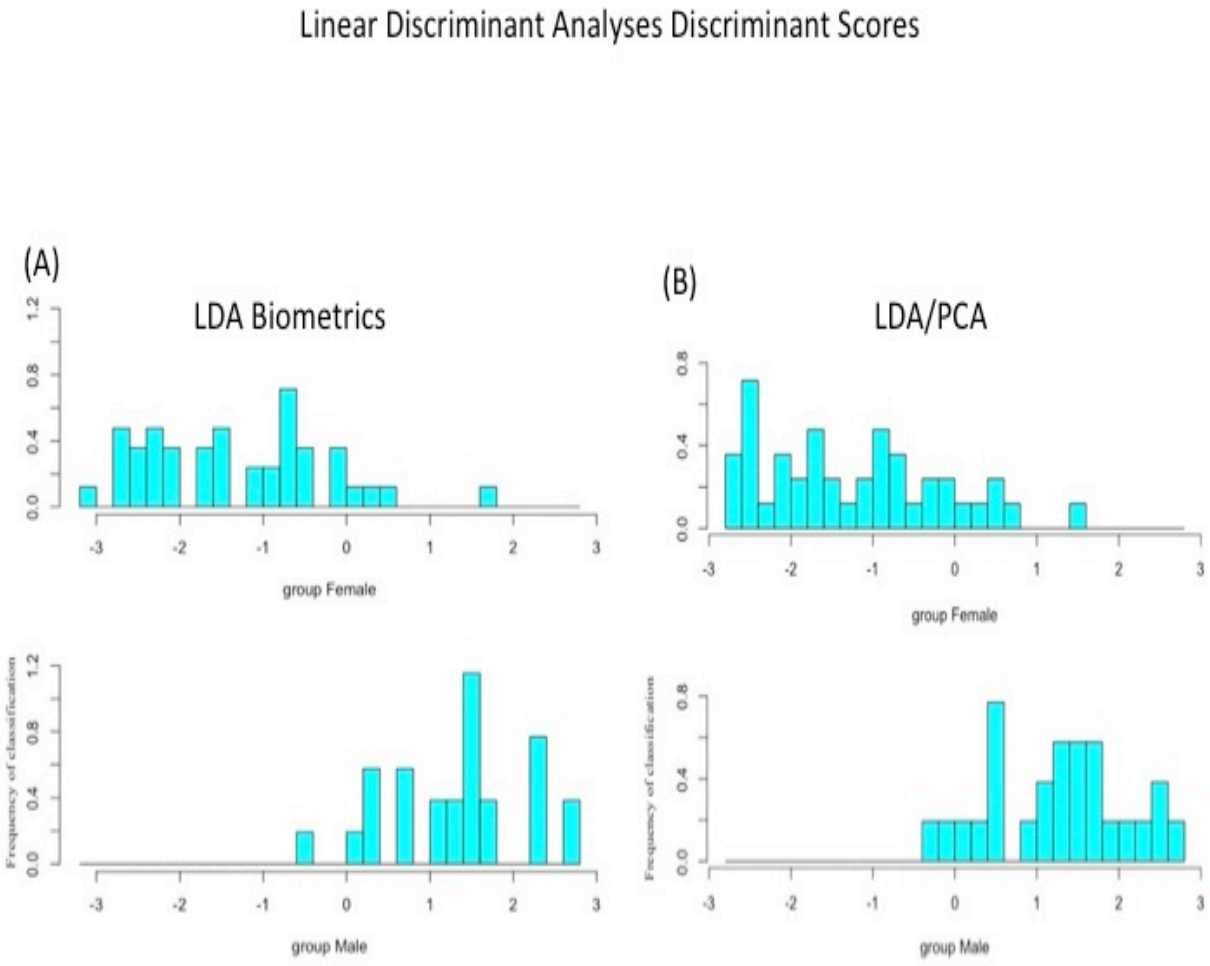

Figure 3.3.1: Distribution of assigned individual discriminant scores by LDA model

The accuracy of the QDA model is 91.2 per cent with a total of 3 males and 3 females misclassified (Table 3.3.1). The cross-validated equivalent of the model had an additional misclassified female with 89.71 percent accuracy. The minor difference between the cross-

validated and non-cross validated equivalent of the model indicates that the classifications of the adult *L. coronata* are stable.

Individuals were frequently misclassified across models. Three individuals, ring numbers: GAA0034, AA0313, and AA0328 were misclassified in each of the DFA models (Table 3.3.2). The females GAA0034 and AA0328 were misclassified due to a wing chord greater than 59.5 millimeters. The male AA0313 was misclassified with a respective head length of 25.8 millimeters and weight greater than 8.9 grams. The 3 repeated misclassified individuals make up 4.23 per cent of the sampled *L. coronata*, indicating that the number of individuals to be misclassified by DFA modelling is minor.

Misclassified *L. coronata*						
Ring Number	Sex	Model	Cross-validated*	Head	Wing	Weight
AA0422	Female	LDA	N	25.4	60	9.1
AA0176	Female	LDA, LDA/PCA	QDA	26	59	8.6
GAA0013	Male	LDA/PCA	LDA	25.93	59	8.5
AA0328	Female	LDA, LDA/PCA, QDA	N	26	60	9.6
GAA0034	Female	LDA, LDA/PCA, QDA	N	26.1	60	9.6
AA0313	Male	LDA, LDA/PCA, QDA	N	25.8	59	9.5
AA0157	Male	LDA, QDA	N	25.4	60	9.5
AA0160	Female	LDA/PCA	Y	25	58	9.3
AA0315	Female	LDA/PCA	N	25.2	59	9.5
AA0422	Female	LDA/PCA, QDA	N	25.4	60	9.1
AA0313	Male	QDA	N	25.8	59	9.5

Table 3.3.2 Biometrics data of *L. coronata* misclassified by DFA model

*Indicates N=not misclassified, Y= misclassified by listed model, in multiple misclassifications model is listed

Section 4: Discussion

The hypothesis of reverse sexual dimorphism in the adult San José de Payamino population of *L. coronata* has been accepted. The sexual size dimorphism calculation indicates that males have significantly smaller total head and weight biometric measurements, consistent with the literature on Pipridae species of similar size (Payne 1984; Théry 1997; Kirwan & Green 2011). Female *L. coronata* are larger bodied than males with a distinguishing weight larger than 8.96 grams and a total head size greater than 25.85 millimeters.

The San José de Payamino population of *L. coronata* differs from the observed trends of reverse sexual size dimorphism in wing chord measurements. The uncharacteristic long wing chord of 59.5 millimeters or greater of male *L. coronata* does not follow the trends for Pipridae of similar size observed by Payne (1984). A study of wing-shape variation, inclusive of *Lepidothrix serena,* by Théry (1997) suggests that a longer wing arm of smaller Pipridae males may increase mobility in lek displays. The larger wing chord observed in male *L. coronata* may be a sexual selection mechanism to increase flight ability for courtship display; however, there is currently insufficient evidence to reach a scientific conclusion on the hypothesis. For the purpose of this research, the sexual size dimorphism of head, weight, and wing chord in the San José de Payamino population has clearly indicated there are physical traits that differ between the sexes that can be applied to create sexing models.

The tree classification analysis provided a graphical representation of the natural subdivisions present in the biometrics of definitively plumaged *L. coronata.* The reverse sexual dimorphism of head and weight measurements and male biased wing chord provide a clear natural division that could be used as a field tool for sexing neotenic males and

indistinguishable females. The applicability of head, wing chord, and weight biometric divisions in classification trees as a field key for sexing will depend on further testing.

The comparison of DFA modelling methods for the purpose of biometric sexing has proven that discriminant function analysis is a successful sexing method. The three modelling methods proved to be comparable, with the lowest classification accuracy occurring in the LDA/PCA model at 89.06 per cent and the highest accuracy of 92.86 per cent for the LDA biometrics model. While the linear biometrics model had the highest accuracy, the quadratic discriminant model provided a near equivalent classification accuracy of 91.2 per cent.

The classification accuracies of the discriminant models is consistent with the literature on discriminant sexing of avian species, where published studies with lower levels of classification accuracies start at 70 per cent and high levels of classification accuracies are typically above 85 per cent (Arizaga et al. 2008; Bluso et al. 2006; Ryder & Durães 2005; Puebla-Olivares & Figueroa-Esquivel 2009). The results produced by the three tested DFA models are consistent with the results observed in literature.

The linear and quadratic models employing biometric measurements as input variables had consistent classification results in the cross-validated and non-cross validated model equivalents. The consistency of the model results indicates a strong discerning capability and a reproducibility of classification results in future applications. The LDA/PCA had the lowest cross-validated accuracy of 88.23 per cent, making it the worst performing model compared. Due to the decreased comparative reproducibility of the results comparative to the biometric models, the LDA/PCA model will not be used in future research.

The statistical power of the DFA models was limited by the sample size. A total of 68 samples is considered to be a small sample population for discriminant analysis and is less than the typical sample size of published avian sexing results in literature (Dechaume-

Moncharmont et al. 2011). The size of the sample restricts the discerning ability of the models in the context of the larger San José de Payamino population of *L. coronata* by failing to account for the extent of biometric variation. Until further testing is conducted, the statistical power and applicability of discriminant modelling of the San José de Payamino population cannot be concluded. While smaller samples are not preferred due vulnerabilities in overestimating or underestimating discriminantion abilities, it is possible to make an assessment of model performance based on model construction.

The validity of discriminant model categorisation is also assessed by the elements of the model construction and the satisifaction of design criteria. The ratio of input variables to individuals categorised is preferenced at 20 individuals to 1 variable (Dechaume-Moncharmont et al. 2011; Spicer 2005). The minimum sample size is determined by the category with the smallest number of individuals, which must be greater than the number of input variables with at least 20 individuals preferred (Dechaume-Moncharmont et al. 2011; Spicer 2005). The ratio used in the DFA analyses had 22 individuals to a single variable (68:3). The smallest group category was male *L. coronata* (n=26) is larger than the number of input variables (n=3) and is over the preferred 20 records. Additional requirements for linear discriminant analysis include testing data for normality and eliminating issues such as outliers.

The definitive plumaged *L. coronata* data met the criteria to perform discriminant analysis despite the small sample. The sensitivity of linear discriminant analysis to outliers was adequately assessed by careful data screening and the inclusion of quadratic analysis, which does not share the same sensitivity to data abnormalities (Lachenbruch et al. 1973; Nakanishi & Sato 1985; Pohar et al. 2004). The evaluation of discriminant model performance detailed in this research, as a pilot study for future work, has concluded that DFA sexing models are worth pursuing in future studies.

The sexual size dimorphism of mature *L. coronata* has made biometric sexing a considerable option in solving the problem of sexing monomorphic mature birds. Discriminant models have shown tremendous potential to be used as a sexing method that is accessible, inexpensive, and is compatable with the restrictions of working in isolated field conditions. Further testing of a larger sample will allow better assessment of the performance and accuracy of linear and quadratic DFA sexing in reflection of population variation. In future research, the decision in selecting between an LDA and QDA method will be determined based on the data structure, as the classification accuracies are comparable.

Section 5: Conclusion

The research conducted on the biometric sexing of *L. coronata* at the Timburi Cocha Scientific Research Station has positively indicated that discriminant function analysis can be used successfully with significant accuracies. Models constructed with biometric variables produced the best sex classifications. In future research, discretion will be taken whether to work with a linear or quadratic model based on data normality. The classification tree graph may have field sexing applicability for mature *L. coronata* in monomorphic plumage by illustrating divisions in biometric measurements due to reverse sexual size dimorphism and male wing chord bias. Currently, the 2013 University of Glasgow Field Course is putting a classification tree to the test for utility as an identification key.

Unfortunately, the application of DFA to the problem of sexing mature monomorphic *L. coronata* could not be accurately assessed during the time frame of this research. Over the 13-year collection period, a total of 3 monomorphic plumaged individuals have been recaptured and sexed by definitive sexual characteristics such as plumage or iris colouration. Furthermore, molecular sexing was not possible due to the restrictive biological sampling and prohibitive genetic laws put in place by the Andean Council in Decision 391 (The Commission of the Cartagena Agreement 1992). The comparison of DFA models detailed in this research was intended to lay down the foundation for the modelling of monomorphic individuals verified by molecular sexing.

Since the end of the research conducted for this study, the Universidad Estatal Amazónica (UEA) in collaboration with the University of Manchester has assumed the maintenance and management of Timburi Cocha Research Station. The involvement of the UEA will facilitate permits for genetics sampling to become assessable. It is intended to

expand upon the current findings using genetic sexing to allow for discriminant models to be comprehensively tested for accuracy sexing monomorphic mature *L. coronata*.

Audio lures will continue to be used in future field seasons, which will enable higher capture rates and a greater sample for further model evaluations. Modelling accuracies will be reassessed with the inclusion of novel data to the existing linear and quadratic models. A scientific conclusion will be reached on the use of the DFA models in future study beyond evaluating the potential of the sexing method as determined in this research. Additionally, the inclusion of tail and interpubic biometric measurements has begun to be instigated for *L. coronata* to add further discrimination potential to the models. Interpubic distance has been employed successfully in Pipridae species with LDA modelling to sex definitive individuals (Mendenhall et al. 2010) and may provide better sex discrimination of monomorphic individuals.

Other future research plans include an extensive study of lekking courtship behaviours, expansion upon novel biometric measurement collection, and the continuation of a photographic record. During the course of 2012 and 2013 field season, *L. coronata* leks were observed. The single full courtship display observed appears to significantly differ from the behaviours documented by Durães (2009), who conducted research on *L. coronata* courtship behaviours elsewhere in the Orenalla Province of Ecuador. The display observed included bowing, shuffling, bobbing and a unique vocalization that are not known aspects of the *L. coronata* repertoire. Further study is planned to record these behaviours and validate courtship differences between the Orenalla populations.

A photographic record of captured *L. coronata* wings, colour bands, and eye colouration was instigated over the 2013 field season to provide a visual record of moult and plumage. The photographs were compiled to add a visual record to the extensive data

collected on *L. coronata* and will be compared to photographic records from previous research conducted by Dr White. In subsequent studies of physical characteristics to sex monomorphic *L. coronata* the photographs may provide insight to nuances in plumage colouration and the redness of the iris to aid in creating a sexing method for monomorphic birds.

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Appendices

Appendix A: Adult Data

Ring Number	Sex	Bill	Head	Tarsus	Wing	Weight	Year
GAA0042	Male	10.38	25.26	13.4	59	9	2002
GAA0041	Male	9.65	25.19	13.19	58	8.8	2002
GAA0036	Female	10.75	25.59	14.31	60	9.5	2002
GAA0034	Female	9.19	25.42	16.35	59	9	2002
GAA0022	Female	9.49	27.02	12.32	59	9.9	2002
GAA0021	Female	10.08	27.06	13.93	57	9.7	2002
GAA0015	Female	10.1	26.41	14.28	56	10.5	2002
GAA0013	Male	10.98	25.93	15.92	59	8.5	2002
GAA0010	Female	10.67	25.61	14.16	56	9	2002
GAA0008	Female	11.95	27.05	14.03	55	9	2002
AA1136	Male	9.1	25	13.6	62	7.6	2012
AA1106	Female	10.2	26.7	13.2	60	11.5	2012
AA1100	Male	9.5	24.7	13.9	60	8.5	2012
AA0856	Female	11.3	26.1	13.2	61	10.2	2009
AA0853	Male	8.5	25	13.3	62	7.9	2011
AA0843	Male	11.9	24.4	12	59	7.5	2008
AA0832	Male	9.5	24.9	13.4	60	8.4	2008
AA0696	Male	10.3	25.3	14.4	60	8	2009
AA0687	Female	10.1	26.4	14.4	60	9.6	2009
AA0540	Female	10.4	26.5	15	59	9.2	2007
AA0536	Female	10.3	27	14.3	59	10.9	2007
AA0532	Female	10.6	27.1	14.6	60	9.4	2007
AA0522	Female	10.8	26.5	15.4	57	10.5	2007
AA0448	Female	8.8	28.5	13.6	60	10.5	2006
AA0443	Female	7.8	26.6	13.5	58	10.5	2006
AA0440	Female	8.1	26.8	13.7	58	10.4	2006
AA0436	Female	11.1	26.7	12.3	59	10.1	2006
AA0435	Female	10.6	26.9	13.3	58	10	2006
AA0432	Female	10.7	27	14.2	60	9.9	2006
AA0422	Female	9.1	25.4	14.4	60	9.1	2006
AA0421	Female	9.83	26.35	14.2	60.25	9.63	2006-2012
AA0342	Female	9.7	25.4	15.1	58	10	2005
AA0341	Female	10.3	27	13.6	60	11	2005
AA0339	Male	8.6	24.8	16.2	60	8.5	2005
AA0333	Male	9.1	24.7	12.7	61	8	2005
AA0329	Female	7.7	25.7	15.7	58	9.5	2005
AA0328	Female	10.1	25.1	12.8	62	8.5	2005
AA0325	Female	9.5	25.9	13.6	58	9	2005

Ring Number	Sex	Bill	Head	Tarsus	Wing	Weight	Year
AA0319	Female	10.2	26.6	15.3	59	10	2005
AA0315	Female	9.1	25.2	16.1	59	9.5	2005
AA0313	Male	9.4	25.8	16.8	59	9.5	2005
AA0312	Female	9.1	25.5	14.4	62	NA	2005
AA0311	Female	11.3	27.7	12.8	59	9.7	2005
AA0177	Male	10.1	25.5	14	62	9.5	2003
AA0176	Female	8.3	26	12.5	59	8.6	2003
AA0172	Female	10.4	27	12.7	59	9.9	2003
AA0165	Female	10.9	27.1	14.1	58	10.4	2003
AA0160	Female	10.3	25	12	58	9.3	2003
AA0157	Male	8.5	25.4	15.3	60	9.5	2003
AA0149	Female	10.3	26.5	16.8	61	9.7	2003
AA0148	Female	8.4	26.3	15.8	56	NA	2003
A1026	Male	10.1	25.8	14.5	60	8.4	2011
A1013	Female	9.5	26.2	14.8	59	10.2	2011
A1004	Female	9.4	26.4	14.7	59	9.6	2011
A1000	Female	10.1	27.25	14.2	59	10.75	2011-2012
A0999	Male	9.2	24.6	13	63	8.2	2011
A0997	Female	7.9	26.1	14.1	60	9.6	2011
A0988	Male	9.55	25.45	14.2	61.5	8.5	2011-2012
A0982	Male	10.2	24.95	13.95	61	9.175	2011-2012
A0978	Male	8.75	24.88	12.85	59.25	8.93	2011-13
A0971	Female	10.6	26	13.8	57	9.9	2011
A0964	Male	9.43	24.47	13.2	60	8.43	2011-2013
A0959	Male	9.13	25.33	14.23	61.5	8.68	2011-2012
A0951	Female	10.1	25.9	15.7	59	9.5	2011
A0918	Male	8.4	25.7	14.3	62	8.4	2009
A0913	Female	10.3	26.9	14.1	59	10.4	2011
A0907	Male	10.2	24.9	15	62	8	2009
A0814	Male	9.8	25.1	13.4	61	8.5	2009
A0811	Male	11.9	25.1	16.5	60	8.6	2009
A0801	Male	9.5	25.5	15.1	60	NA	2009
A0696	Male	8.6	24.6	13.8	60	8.4	2008

Appendix B: Immature Data

Ring Number	Sex	Bill	Head	Tarsus	Wing	Weight	Year
A0767	Not Adult Male	10	27.3	12.9	58	9.8	2009
A0768	Not Adult Male	9.7	26.4	14.7	61	10.2	2009
A0770	Not Adult Male	10.5	26.9	15.8	58	9.7	2009
A0775	Not Adult Male	10.9	27.3	14.8	57	9.8	2009
A0791	Not Adult Male	9.8	26.9	14.7	59	9.7	2009
A0799	Not Adult Male	10.9	26.5	14.6	59	10	2009
A0802	Not Adult Male	10.8	26.8	15	60	10.7	2009
A0904	Not Adult Male	10.7	25.7	14.3	56	10	2009
A0905	Not Adult Male	10	24.7	16.1	59	9.3	2009
A0910	Not Adult Male	10	26.4	14.9	59	9.7	2009
A0911	Not Adult Male	9.9	24.4	15	58	8.4	2009
A0913	Not Adult Male	10.92	27.18	15.37	58.17	10.4	2009, 2011, 2012
A0915	Not Adult Male	9.8	25.2	16.2	60	9.4	2009
A0928	Not Adult Male	10.5	27.5	15.5	60	10.8	2009
A0933	Juvenile	10.8	27	14.3	58	9.5	2011
A0937	Juvenile	10	26.5	14.7	59	9.6	2011
A0939	Juvenile	10.55	27.5	14.9	59.5	9.95	2011
A0940	Juvenile	9.4	24.9	14.6	59	8.4	2011
A0945	Juvenile	9.6	24.8	15	60	9.4	2011
A0948	Juvenile	9.8	25.2	14.1	59	9.6	2011
A0952	Juvenile	9.1	25.5	14.8	63	10.3	2011
A0958	Juvenile	10.9	26.2	14.3	57	9.4	2011
A0972	Juvenile	8.3	25.2	12.9	60	8.9	2011
A0975	Not Adult Male	9.6	26.9	14.1	60	10	2012
A0979	Juvenile	9.9	26.8	16.5	57	10.2	2011
A0985	Juvenile	10.7	25.4	15.2	59	9	2011
A1009	Juvenile	9.6	23.7	14.4	61	7.9	2011
A1019	Juvenile	10.2	25.8	13	57	9.4	2011
A1022	Juvenile	10.6	26.8	13.8	58	9.9	2011
AA0415	Not Adult Male	9.2	26.3	12.5	55	9.3	2006
AA0687	Not Adult Male	9.9	26.7	13.8	60	9.9	2008
AA0692	Juvenile	9.3	26.9	13.7	60	10.3	2008
AA0700	Not Adult Male	9.9	25.9	14.3	59	11.1	2008
AA0701	Not Adult Male	8.8	26.1	14.7	60	7	2008
AA0702	Not Adult Male	9.2	25.1	15.4	63	9.6	2008
AA0707	Juvenile	9.2	26.3	14	60	8.1	2008
AA0818	Not Adult Male	10.8	26.7	14	59	9.8	2008
AA0820	Not Adult Male	10	26.4	16.3	56	10.2	2008
AA0831	Not Adult Male	10.7	27	13.6	59	9.7	2008
AA0856	Not Adult Male	11.6	25.6	11.8	63	10.5	2008
AA0857	Not Adult Male	10.2	26.8	13.6	57	11.3	2008
AA1084	Not Adult Male	10.4	26.9	15	60	10.3	2012
Ring Number	Sex	Bill	Head	Tarsus	Wing	Weight	Year

AA1086	Juvenile	10.1	25.4	15.6	59	8.9	2012
AA1107	Juvenile	9.5	24.5	13.8	59	8.2	2012
AA1108	Juvenile	9.5	24.5	13.8	59	8.7	2012
AA1113	Juvenile	9.3	25.3	13.7	60	8.2	2012
AA1116	Juvenile	11.2	28	13.7	59	10.93	2012
AA1119	Juvenile	9.2	25.6	14.1	58	9.53	2012
AA1122	Not Adult Male	10.35	27	12.1	61	10.43	2012-2013
AA1128	Juvenile	10.5	27.2	15.5	61	9.9	2012
AA1141	Juvenile	10.8	27.2	13.5	60	10.05	2012
AA1142	Juvenile	10.9	26.9	13.5	59	10.2	2012
Purple/black	Juvenile	10.3	25.4	10.3	58	9.7	2013
Purple/blue	Not Adult Male	10.1	26.6	12.5	61	9.6	2013
Purple/Green	Not Adult Male	10	26.3	10.6	55	9.5	2013
Purple/purple	Juvenile	9.5	25	11.6	58	9.7	2013
Purple/red	Juvenile	10.1	25.7	12.8	57	9	2013
Purple/white	Juvenile	10	26.8	11.8	55	10.1	2013
Purple/yellow	Not Adult Male	10.4	26.3	13.1	56	10.1	2013
White/black	Not Adult Male	9.7	25.5	13.2	56	8.8	2013
White/blue	Juvenile	10.1	25.4	13.4	59	9.4	2013

Appendix C: Pilot Linear Discriminant Model of Biometrics

The pilot linear discriminant analysis (LDA) of the adult *L. coronata* classified 88.23 per cent of individuals accurately. A total of 92.31 per cent of males and 92.86 per cent of females were classified accurately, with 3 males and 2 females misclassified (Table C1). The corresponding discriminant equation: 0.05(bill) + 0.06(tarsus) + 0.30(wing) – 0.78(head) – 0.88(weight) indicated that head and weight were the strongest contributing variables to discerning individuals to sex category. The sign of the linear coefficients were indicative of group separation and a larger coefficient indicated variable significance the variable to the assignment of discriminant scores.

Classification Results of Pilot LDA Biometrics Model			
	Male	Female	Total
Male	24	3	27
Female	2	39	41
Total	26	42	68

Table C1: Mature *L. coronata* assignment to sex category LDA biometrics

The discriminant scores calculated by the LDA equation produced a clear division between male and female birds, which was represented graphically with a histogram (Figure C1). Males are depicted on the bottom histogram and females are depicted on the top. The male group was assigned predominately negative discriminant scores while the female group was assigned positive discriminant scores. The distribution of the discriminant scores and the high accuracy of sex classification of adults indicated that linear discriminant analysis conducted directly on biometrics measurements was an effective means to classify adult *L. coronata*.

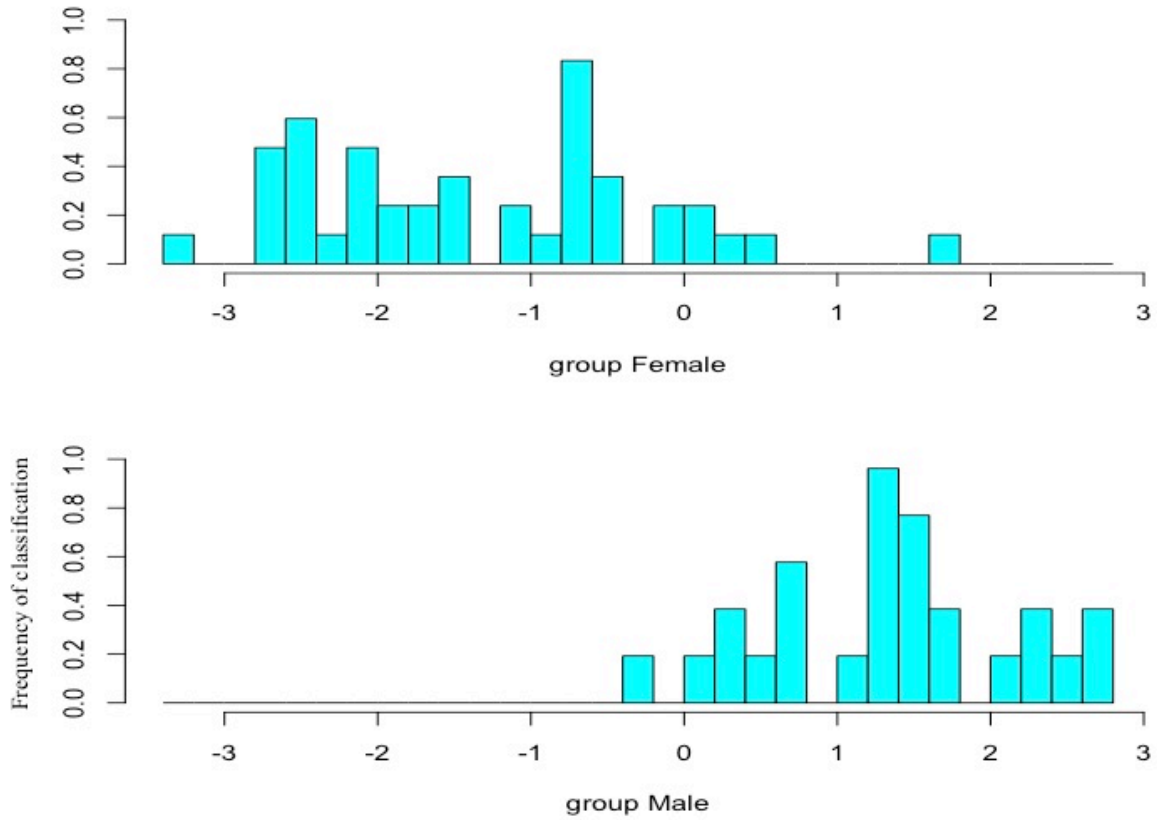

Figure C1: Histogram of discriminant score distributions pilot biometrics LDA model

The partition plot of the discriminant analysis results indicated the interaction of variables in determining accuracy classifications (Figure C2). The density of males is indicated in the pink tone, while the density of females in the sample is indicated by blue (Figure C2). The first letter depicts the sexes of individuals, with misclassifications indicated in red. The error rate associated with variable parings are indicated on top of each individual plot, with the lowest error rates associated with variables determined to be significantly dimorphic in males and females. The lowest error rate of 10.3 per cent belonged to the partition plot of weight and wing chord, while the second lowest error rate of 13.2 per cent belonged to the plot of head and weight (Figure C2).

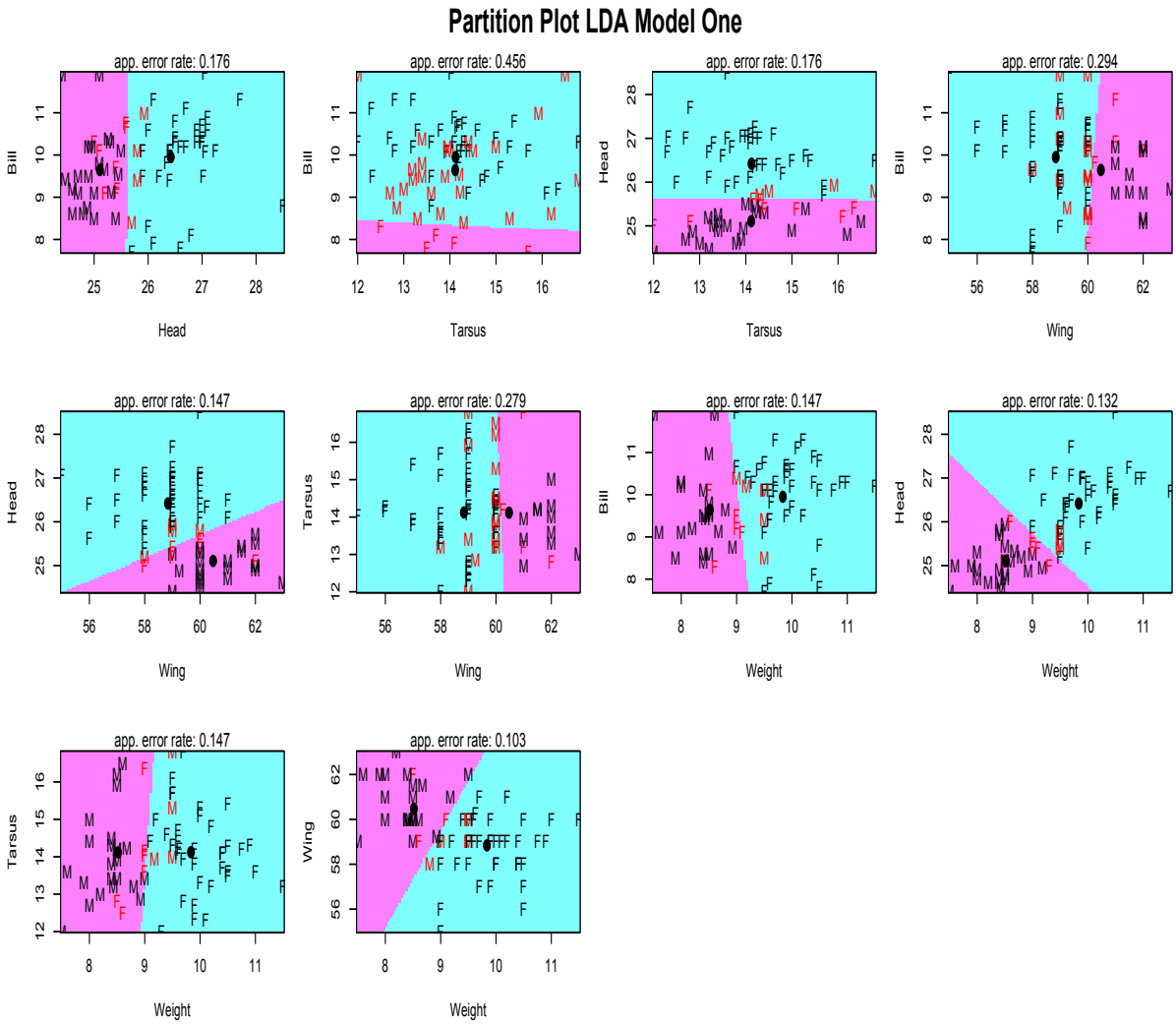

Figure C2: Variable error associated with biometric measurements in a biometrics LDA model

The ability of the model to classify adult *L. coronata* was evaluated through the use of leave one out cross-validation. The cross-validated equivalent of the model produced very similar results to the non-cross-validated model, with an increase in the number of misclassified females. A total of 5 females were misclassified as male, an increase from 2 misclassified females, giving the model an overall misclassification rate of 11.76 per cent (Table C2). The minor differences in classification accuracy between the cross-validated and non-cross validated equivalents indicate the robustness of the linear discriminant model to correctly classify adult *L. coronata.*

Cross-Validated Classification Results			
	Male	Female	Total
Male	23	3	26
Female	5	37	42
Total	28	40	68

Table C2: LDA biometric classification with leave one out cross-validation

Appendix D: Pilot LDA Model of Principal Components

The principal component analysis of the adult *L. coronata* data, like the prior analysis conducted on the total data set of *L. coronata*, had five principal components to match the number of variables to the total variation. The first three principal components explained the majority of the variance, with the first principal component accounting for 47 per cent of the data variation (Table D1). The second principal component added an additional 21 per cent explanation and the third principal component another 16 per cent of explained variation (Table D1). Cumulatively, the three principal components explained 84 per cent of the variation within the data set. The Kraiser Criterion indicated that the first two principal components were the most significant explanations for the variation found within the data set.

Principal Component Analysis of Adult *L. coronata*					
	PC1	PC2	PC3	PC4	PC5
Standard Deviation	1.72	1.14	0.99	0.92	0.42
Proportion of Variance	0.47	0.21	0.16	0.14	0.03
Cumulative Proportion	0.47	0.68	0.84	0.92	1.00

Table D1: Variation of adult biometrics explained by principal components

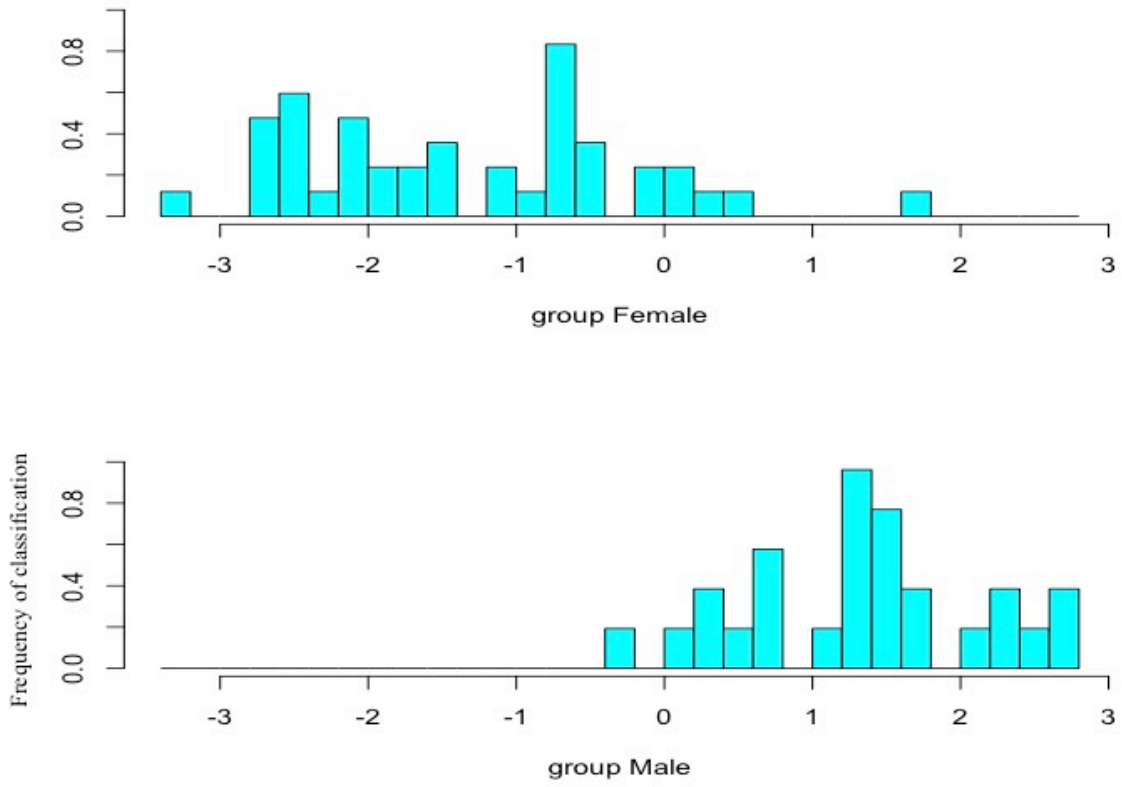

Figure D1: Histogram of discriminant scores of LDA/PCA model

The principal components were used to create the second pilot linear discriminant model. The initial analysis employed all five principal components, allowing all variation to be used to establish a base model to be simplified. The model classified a total of 92.65 per cent of adult *L. coronata* accurately, producing identical results to the prior non-simplified LDA model (Table D1).

Classification of Adult *L. coronata* Pilot LDA Principal Components Model			
	Male	Female	Total
Male	24	2	26
Female	3	39	42
Total	27	41	68

Table D1: Classification Pilot LDA/PCA Model

The histogram of the discriminant scores of the second LDA model produced significantly different discriminant score distributions than what were produced in the first pilot LDA model (Figure D1). The top histogram depicts female *L. coronata* discriminant scores, which are shifted closer to center than the first pilot model histogram. The female discriminant scores are still predominately negative, but the frequencies of small negative discriminants scores have noticeably increased (Figure D1). The bottom histogram depicts the male *L. coronata,* which have shifted similarly to female scores toward the center and zero value. The overall discriminant scores for males are predominately positive, but with an increased frequency of scores closer to zero.

The partition plots of the second LDA model indicate the interactions of the principal components in determining sex classifications in the analysis (Figure D2). The partition plots of the adult principal components have an overall higher relative error rate than the partition plots produced from the biometric measurements in the previous appendix. For the majority of the plots, most of the misclassified individuals were males. Despite the overall higher error rate, the plot of the first and third principal components had the lowest observed relative error of 8.8 per cent (Figure D2). The next lowest relative error plots also included the first principal component, with the plot with principal component two having a 14.7 per cent error and the plot with principal component four having a 16.2 per cent error (Figure D2).

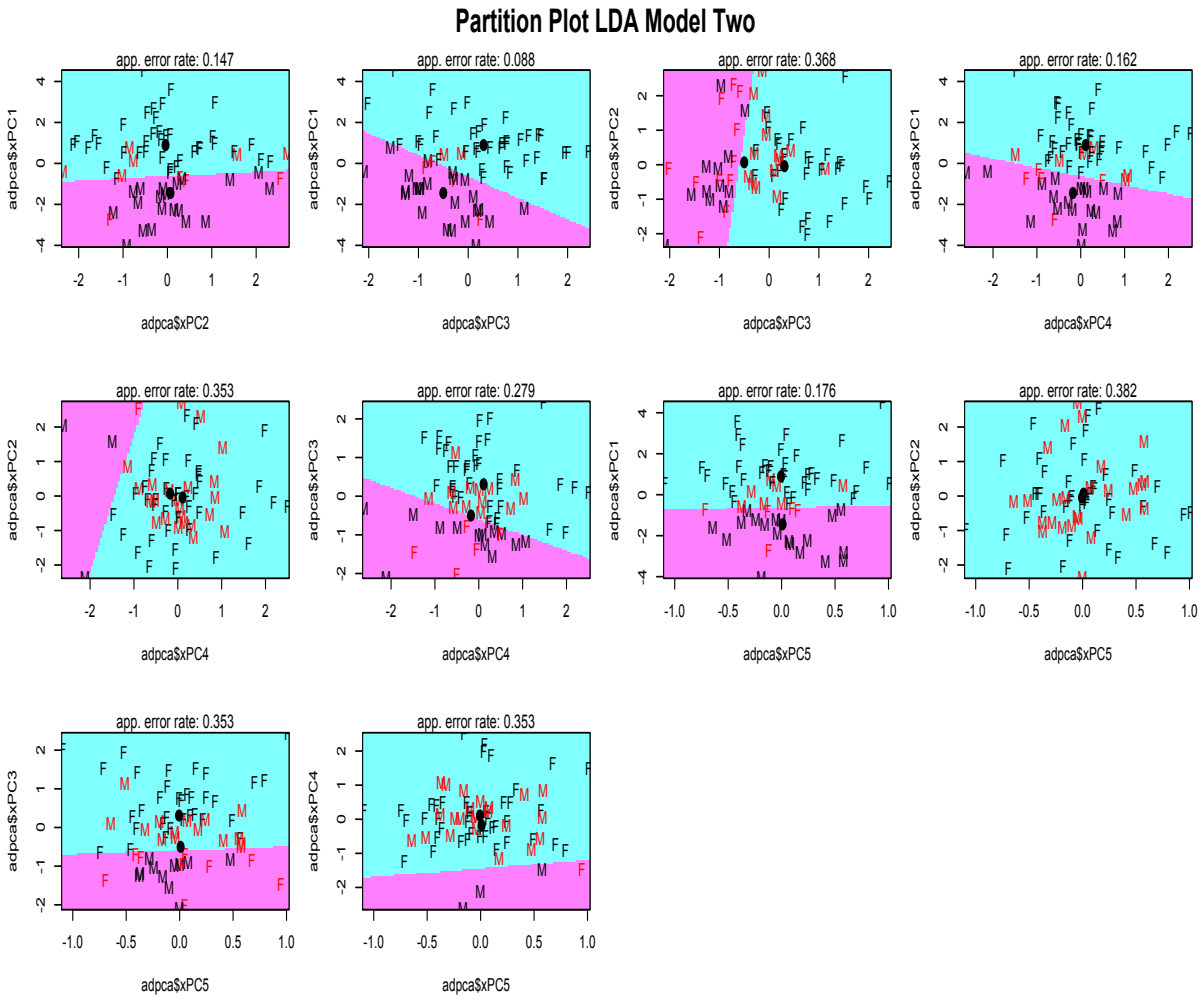

Figure D2: Partition plots of the pilot LDA/PCA model

The cross-validated equivalent of the second model, as observed in the prior model, had a slightly larger misclassification of males and females. A total of 5 females were misclassified as male and 3 males were misclassified as female (Table D2). The classification accuracy of the cross-validated model dropped from 92.65 per cent to 88.24 per cent, indicating minor classification differences.

Classification Table of Cross-Validated Pilot LDA Model Two			
	Male	Female	Total
Male	23	3	26
Female	5	37	42
Total	28	40	68

Table D2: Cross-validated classifications pilot LDA/PCA model

Appendix E: Pilot Quadratic Discriminant Model

The QDA of adult *L. coronata* biometrics produced a high classification accuracy rate of 94.12 per cent (Table E1). The classification accuracy of the QDA model was approximately 2 per cent higher than the first LDA model used biometrics in section 5.8.1. A total of 1 male were misclassified, giving male classification an accuracy of 96.15 per cent (Table E1). Three females were misclassified as male for classification accuracy of 92.86 per cent (Table E1).

Predicted Classification of Adult *L. coronata* for Pilot QDA Model			
	Male	Female	Total
Male	25	1	26
Female	3	39	42
Total	28	40	68

Table E1: Predicted sex of pilot QDA model

The partition plot of the QDA model had numerous variable combinations with the same relative error (Figure E1). The lowest error rate combination shared by the head and weight plot and the plot of wing and weight which had an 11.8 per cent error rate (Figure E1). The significant variables determined in the SSD calculation, head, weight, and wing produced the partition plots with the lowest relative error. The second lowest relative error was 14.7 per cent, which was shared between four partition plots where head, weight, and wing were at least one of the variables (Figure E1).

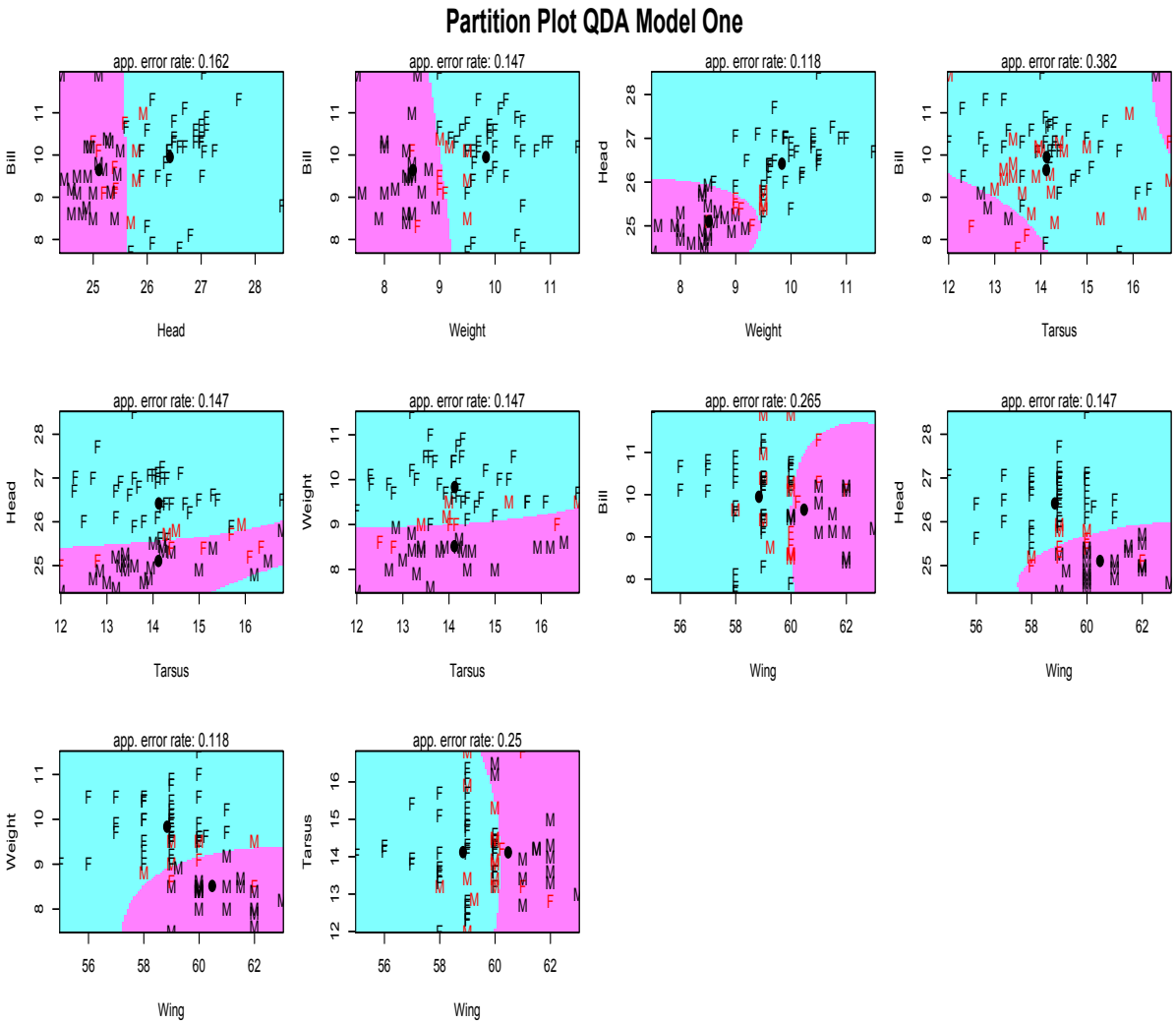

Figure E1: Partition plot of pilot of the QDA model

The classification of the cross-validated equivalent of the model had a significant drop in classification accuracy. The cross-validated model had a 19.12 per cent misclassification rate, an increase of approximately 14 per cent from the non-cross-validated equivalent (Table E2). The misclassification of male birds noticeably increased from a single individual to 7 with the cross-validated equivalent (Table E2). The misclassification of females also increased from 3 individuals to 6 total (Table E2).

Classification for Cross-Validated Pilot QDA Model			
	Male	Female	Total
Male	19	7	26
Female	6	36	42
Total	25	43	68

Table E2: Cross-validated classification pilot QDA model