PLUMAGE COLORATION AND MORPHOLOGY IN *CHIROXIPHIA* MANAKINS:

INTERACTING EFFECTS OF NATURAL AND SEXUAL SELECTION

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PLUMAGE COLORATION AND MORPHOLOGY IN *CHIROXIPHIA* MANAKINS: INTERACTING EFFECTS OF NATURAL AND SEXUAL SELECTION

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DISSERTATION ABSTRACT

PLUMAGE COLORATION AND MORPHOLOGY IN *CHIROXIPHIA* MANAKINS: INTERACTING EFFECTS OF NATURAL AND SEXUAL SELECTION

Stéphanie M. Doucet

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I examined how natural and sexual selection may have influenced the morphology and coloration of *Chiroxiphia* manakins (Aves: Pipridae). In the first chapter, I investigated age– and sex–related patterns of plumage coloration and molt timing in long–tailed manakins, *C. linearis*. I examined how plumage coloration changed with age in males and females based on 1315 mist net captures in northwestern Costa Rica and examined molt patterns in an additional 585 museum specimens. Males followed a remarkably age–specific pattern of plumage maturation before attaining definitive adult plumage in their fifth year. Some females developed male–like plumage characteristics as they aged, but not reliably so. Although molt–breeding overlap occurred at the population level, few individuals molted while breeding. In the second chapter, I examined the explanatory power of natural and sexual selection hypotheses in explaining patterns of

sexual dimorphism in *Chiroxphia* manakins. I measured six morphological traits in 361 wild *C. linearis* and a subset of these traits in 872 museum specimens of *Chiroxphia* manakins. My findings were consistent with both sexual selection and natural selection hypotheses, suggesting that both mechanisms have strongly influenced the evolution of sexual dimorphism in this group. In the third chapter, I investigated how the perceptual environment influences the conspicuousness of plumage displays in long–tailed manakins. I measured the reflectance of 62 males and 59 females, the reflectance of the background vegetation, and the irradiance of light at display perches. Male manakins were highly conspicuous against the visual background, whereas females were relatively cryptic. Males did not appear to adjust the location or timing of their displays to take advantage of particular light conditions but rather displayed in the most common light environment of forest shade. Displays performed in forest shade may optimize the short–distance conspicuousness of male plumage ornaments while minimizing the long–distance conspicuousness of male ornaments and the short– and long–distance conspicuousness of female plumage patterns. In the fourth chapter, I evaluated the degree to which the color of study skins is representative of coloration in wild birds. I measured the plumage reflectance of 58 wild and 55 museum specimens of long–tailed manakins. I found significant differences in color between museum specimens and wild birds, and the degree of difference depended on the coloration mechanism. Potential sources of these differences include the specimen preparation process, the age of specimens, and geographic variation. Although caution is warranted for some types of studies, most differences were relatively subtle, justifying the use of museum specimens to assess color in many instances.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 4

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 1. PLUMAGE DEVELOPMENT AND MOLT IN LONG–TAILED MANAKINS: VARIATION ACCORDING TO SEX AND AGE

ABSTRACT

Lek–mating long–tailed manakins (*Chiroxiphia linearis*) exhibit an unusual pattern of delayed plumage maturation. Each year, males progress through a series of predefinitive plumages before attaining definitive plumage in their fifth calendar year. Females also exhibit variation in plumage coloration, with some females displaying male–like plumage characteristics. Using data from mist net captures in northwestern Costa Rica (n = 1,315) and museum specimens from throughout the range of long–tailed manakins (n = 585), we document the plumage sequence progression of males, explore variation in female plumage, and describe the timing of molt in this species. Males progressed through a series of age–specific predefinitive plumages, enabling the accurate aging of predefinitive–plumaged males in the field and providing the basis for age–related status signaling in these males. Females tended to acquire red coloration in the crown as they aged. However, colorful plumage in females may result as a by–product of selection on bright male plumage. Females exhibited an early peak of molt activity from February to April, little molt from May through July, and a second, more pronounced peak of molt activity in October. By contrast, males in older predefinitive plumage stages and males in definitive plumage exhibited comparable, unimodal peaks in molt activity beginning in June and peaking between July and October. Our data are consistent with selective pressure to avoid the costs of molt/breeding overlap in females and older males. Our findings have important implications for social organization and signaling in long–tailed manakins, and the evolution of delayed plumage maturation in birds.

INTRODUCTION

In many birds, males delay assuming definitive, adult–like plumage for one or more years after hatching. In some species, this delay in plumage maturation is accompanied by a delay in sexual maturation (Lawton and Lawton 1986). In many sexually dichromatic, north–temperate passerines, however, males delay attaining their definitive plumage until after their first potential breeding season, despite having reached sexual maturity (Rohwer et al. 1980; Lyon and Montgomerie 1986). The adaptive significance of this type of delayed plumage maturation has been the focus of extensive research in recent decades (e.g., Rohwer et al. 1980; Lyon and Montgomerie 1986; Hill 1996), with most studies investigating one–year delays typically exhibited by north–temperate passerines. However, many tropical passerines deviate from this pattern. In some species, such as bowerbirds and birds of paradise, males molt into the same predefinitive plumage for several years before assuming definitive plumage (Frith and Beehler 1998; Frith and Frith 2004). In other species, for example *Chiroxiphia* manakins, *Geospiza* Darwin's finches, paradise flycatchers, Hawaiian honeycreepers, and monarch flycatchers, males progress through a transitional series of different predefinitive plumage stages before assuming definitive plumage (Foster 1987; McDonald 1989a; McDonald 1993a; Lepson and Freed 1995; Vanderwerf 2001; Mulder et al. 2002). Investigations of the adaptive significance of delayed plumage maturation, and of the signal function of variation in plumage, require a thorough understanding of plumage development and molt. Here, we investigate age– and sex–related plumage variation in the long–tailed manakin (*Chiroxiphia linearis*).

Long–tailed manakins have a lek–based mating system (Foster 1977; McDonald 1989a; McDonald 1989b). Males gather at lek sites where they establish age–graded dominance hierarchies. The two most dominant males at each lek, the alpha and beta males, perform vocal duets and elaborate, dual–male dance displays for females visiting the leks. Females usually copulate only with alpha males (Foster 1977; McDonald 1989b; McDonald and Potts 1994). As in other lekking species (Höglund and Alatalo 1995), females are solely responsible for rearing offspring (Foster 1976).

As they grow older, male long–tailed manakins progress through a series of transitional, predefinitive plumage stages before attaining definitive plumage in their fifth calendar year (Foster 1987; McDonald 1989a; McDonald 1993a). This delay is unusually long for such a small (15–21 g) passerine (Lawton and Lawton 1986). Female long–tailed manakins also vary in plumage color, with some females developing red or tawny crown feathers, a trait thought to be associated with age (McDonald 1989b). Such extensive age– and sex–based variation in plumage may present unique signaling opportunities, particularly in species with complex social organization (Lawton and Lawton 1986). In long–tailed manakins, for example, a male's position within a lek hierarchy may be largely influenced by his age. Thus, transitional plumages that reliably signal a male's age and/or status in the hierarchy would likely reduce the occurrence of costly, escalated encounters between males of low and high status (Foster 1987; McDonald 1993a).

Foster (1987) described predefinitive male plumages in long–tailed manakins as transitioning through several stages along a continuous spectrum, with second–year males being mostly green with a red crown patch and, occasionally, some black on the face, coverts, and body and flight feathers, and third– and fourth–year males having a

mixture of red, green, black, and blue plumage. McDonald (1989a; 1993a) proposed an alternative sequence, whereby males progress through a transitional series of age–specific predefinitive plumages. In his proposed sequence, males develop a red crown in their second year, a black mask in their third year, and a blue mantle in their fourth year. Using recapture and resighting data from three study populations, together spanning 27 years, we reexamine the plumage sequences proposed by Foster (1987) and McDonald (1989a; 1993a) to determine whether predefinitive male plumage can reliably signal age in long–tailed manakins. Additionally, we investigate patterns of variation in female plumage, and describe sex– and age–based variation in the timing of molt.

METHODS

We studied long–tailed manakins at three sites in northwestern Costa Rica: from 1971 to 1974 and 1977 at the Enrique Jiménez Nuñez Experiment Station (10º 20' N, 85º 8' W), from 1981 to 1999 in Monteverde (10˚ 18' N, 84˚ 48' W), and from 2003 to 2005, as well as for a few weeks in 1986, in Santa Rosa National Park, Guanacaste Conservation Area (10˚ 40' N, 85˚ 30' W). The Monteverde site is located in premontane tropical moist forest (Holdridge 1966) and the Santa Rosa and Jiménez Station sites encompass both evergreen bottomland moist forest and areas of tropical dry forest on surrounding hillsides (Stiles and Skutch 1989). All three sites exhibit marked seasonality, with a dry season extending from approximately January through April, and a rainy season extending from May through December. Male long–tailed manakins display actively from February through September, with a pronounced peak in activity from March through June (Foster 1977; McDonald 1989a; McDonald 1989b; McDonald 1993b).

Active nests have been discovered from March to July (Foster 1976; SMD, unpubl. data), although the nesting season likely extends until September (Foster 1976).

We captured 1,315 long–tailed manakins using mist nets and fitted each individual with a unique combination of plastic colored leg bands; birds at Monteverde and Santa Rosa also carried a numbered aluminum leg band. Of 20 birds banded as nestlings, only two were recaptured or resighted in following years (one male and one female). To determine the plumage sequence followed by birds as they aged, we recorded detailed descriptions of each bird's plumage coloration each time it was captured. Whenever possible, we also recorded plumage descriptions of marked individuals seen during behavioral observations at lek sites or encountered opportunistically at the study sites. Over the course of the study, R. P. Clay and M. S. Foster noted that second–year birds of both sexes could be identified by the presence of retained juvenal wing feathers; R. P. Clay also discovered that the mouth–lining color of second–year birds was diagnostic (Clay 2001). Thus, we examined the mouth–lining color and the amount of wear on wing feathers of all green–plumaged birds captured between from 1997 to 1999, and in 2005. Only a subset of the data we present in this study were described briefly elsewhere (plumage sequence information from 56 males, McDonald 1989a; McDonald 1993a).

To assess the timing of molt, we examined all birds captured from 1971 to 1977, some birds captured in 1986 and 1987, and all birds captured since 1995 for signs of molt (i.e., sheathed feathers), recording whether birds were molting at time of capture and noting in which regions of which feather tracts they were molting. Occasionally, we noted extremely asymmetric molt or molt of only a single, isolated feather on some

individuals. We assumed in both instances that the molt was adventitious and did not include these individuals in our calculations of proportions of birds molting (Pyle 1997; Vanderwerf 2001). Although most birds were captured and observed between March and July, we collected molt information on wild birds in all months except September and February.

To obtain additional molt data spanning the calendar year, we also examined 585 long–tailed manakin specimens in the collections of museums listed in the Acknowledgements. We recorded feather regions and tracts with molting feathers and, if discernible, the plumages from which and into which a bird was molting. A large number of the museum specimens were also examined for the presence of juvenal remiges and coverts. Taken together, our data from museum specimens and wild birds spanned the entire calendar year, although some months are considerably better represented than others. Molt and plumage terminology follow the Humphrey–Parkes (H–P) system (Humphrey and Parkes 1959) as summarized in Pyle (1997). A recent review (Howell et al. 2003) recommends modifications to H–P terminology. The complex nature of molt in manakins, however, makes it difficult to assign consistent molt terminology to different sex and age classes under the proposed changes. Thus, for simplicity, we opted for traditional H–P terminology. We also follow Pyle's (1997) age terminology. Thus, a hatch–year is a bird in its first calendar year (until 31 December of the year it fledged), a second–year bird is in its second calendar year (from January 1 to December 31 of the year following fledging), a third–year bird is a bird in its third calendar year, and so on.

Wild birds in all–green or primarily green plumage were identified as females if they had a vascularized brood patch, were recaptured or resighted in green plumage in

multiple years, were aged as after–second year birds based on the absence of retained juvenal wing feathers (see above), exhibited female–like behavior during dance displays by males (McDonald 1989a), or were identified as such either by genetic sexing (Griffiths et al. 1998) or by laparotomy. Birds in green plumage were identified as males if they were recaptured or resighted in predefinitive or definitive male plumages in subsequent years or by laparotomy. For museum specimens, green birds were identified as females or males only if the specimen tag indicated the presence of ovaries or testes, respectively. Birds in green plumage that did not meet these criteria were considered of unknown sex and were not included in our analyses.

RESULTS

Molt

All Long–tailed Manakins underwent a prebasic (postbreeding) molt each year. The olive–green juvenal plumage grown in the nest was identical in males and females. The first prebasic molt began within four months of fledging and was a partial molt, as hatch–year birds of both sexes retained some of their juvenal wing feathers. Thus, second–year birds had two generations of wing feathers: retained juvenal remiges and distal greater coverts and new lesser and median coverts and proximal greater coverts. Additionally, the mouth linings of second–year birds were bright orange to orange–yellow, similar to those of nestlings (Foster 1976), whereas the mouth linings of older birds were paler and more pinkish in color. All subsequent prebasic molts were complete.

Male plumage sequence

Overall, we captured and marked 653 individuals in Monteverde, 280 individuals at the Jiménez Station and 382 individuals in Santa Rosa. Many of these birds were recaptured or resighted in one or more subsequent years, allowing us to document 562 plumage transitions from a total of 235 males. Of these transitions, 343 were successive molts in definitive plumage, while 219 consisted of molts from one predefinitive plumage to another or from predefinitive to definitive plumage. Of these 219 informative transitions, we have records for two different plumage stages for 98 males, three different plumage stages for 32 males, and four different plumage stages for 19 males. One male was sighted in all five plumage stages.

Males progressed through the following plumage sequence (Fig. 1; Tables 1, 2). They acquired their juvenal plumage in the nest, which, like the plumage of females, was olive green above with a paler wash below. Within four months of fledging, males initiated their first prebasic molt. Males in their first–basic plumage, which we term "red–cap plumage", were olive green throughout with a small red crown patch. The amount of red in the crown was highly variable, and males often had two strips of red feathers on the outer edges of the crown rather than a contiguous red crown patch (Fig. 3A). A limited number of males in red–cap plumage also had some black feathers on the face. These black feathers, or additional ones, were acquired along with an expanded red crown in some males during a partial molt (either a limited prealternate molt or the early onset of the second prebasic molt; see *Timing of Molt*) in March and April. In addition, some males undergoing their first prebasic molt replaced their central, but no other,

rectrices (MSF, unpubl. data). These rectrices were darker and longer than those retained from the juvenal plumage.

The following year, males underwent their second prebasic molt. Males in their second–basic plumage, which we term "black–face plumage", were mostly olive–green with a small red crown patch and a black facial mask or hood. In this plumage, the red crown patch was always contiguous and was larger than that of red–cap males but smaller than that of males in definitive plumage. The amount of black on the face varied from a small black facial mask to a full black hood. Some black–face males also had some black in their coverts and flight feathers, and a blackish tinge to their body plumage. Rarely, males in black–face plumage had a limited number of blue feathers on their mantle.

Males in their third–basic plumage, which we term "blue–back plumage", had a mixture of green and black body and flight feathers, a full red crown patch, and some blue feathers on the mantle. Birds in blue–back plumage exhibited the greatest range of variation in proportions of plumage colors, although all exhibited some blue in the back, and some black ventrally. Often, blue–back males had body feathers that were mostly black with a tinge of olive–green and flight feathers that were black with green edging. Some blue–back males had very little green at all in their plumage and might be mistaken for definitive–plumaged males in the field. Other blue–back males had so much green in their body plumage that they had an almost grayish appearance. Finally, males in definitive–basic plumage had entirely jet–black body and flight feathers, a sky–blue mantle, and a bifid, red crown patch. A small number of males molting from blue–black plumage to definitive plumage retained minimal amounts of green on the rump, flanks, or undertail coverts. This green disappeared during the following prebasic molt.

Four lines of evidence allow us to assign ages to these sequences. First, one male banded as a nestling was recaptured or observed in three subsequent years: in his first prebasic molt he acquired the red–cap plumage, in his second prebasic molt, the black–face plumage, and in his third prebasic molt, the blue–back plumage. (Unfortunately, this male was not resighted again until his sixth calendar year, when he was in definitive plumage). Second, all red–cap males captured between 1997 and 1999 at Monteverde (32 individuals) and in 2005 at Santa Rosa (11 individuals) were identified as second–year birds on the basis of retained juvenal remiges and distal greater coverts and mouth lining color. Third, we documented the full, four–year progression of 17 males from red–cap plumage to definitive plumage. Finally, all males observed in red–cap plumage invariably had black feathers on the face the following year. Similarly, all males observed in black–face plumage had blue feathers on their mantle the following year. No male observed in definitive plumage was ever observed to have green feathers in subsequent years. Our observations suggest that this plumage sequence is unidirectional, non–reversible, and remarkably age–specific.

Of the 562 plumage transitions we recorded, 465 were sequential (i.e., the males were recaptured or resighted the following year). These sequential transitions allowed us to assess the frequency of unexpected plumage transitions. We documented only four unusual plumage transitions. Two males were first captured in all green plumage and both were recaptured in black–face plumage the following year (Table 2). Because these males were captured in March and April as green males but the following breeding season as black–face males, it is possible that they showed red in the crown during some of the interval between these records. A third otherwise all–green male was molting in a

limited black mask and red crown when it was captured in April. A year later, after a single prebasic molt, it was in blue–back plumage. A fourth presumably aberrant bird, captured in red–cap plumage and without any black on its body, had a larger red crown, a well developed black hood (but no other black body feathers), and a small area of blue feathers on the back the following year. Thus, at most 0.7 % of plumage transitions deviated from the expected pattern. Even if we exclude transitions between definitive plumage stages, these anomalies account for only 1.8 % of plumage transitions. These anomalies invariably involved an accelerated plumage maturation process, as no male ever remained in the same plumage stage for two subsequent years. In 96 instances, more than one year elapsed between recaptures or resightings of particular males. Even among these non–sequential transitions, males were always in the expected plumage category when they were eventually recaptured or resighted, as estimated from the number of years separating recaptures or resightings.

Female plumage

Female Long–tailed Manakins are typically olive–green above with a paler wash below. However, of the 649 confirmed females examined (including museum specimens), 145 (23 %) had variable amounts of tawny or red feathers on the crown, ranging from a single feather to a full tawny or red crown. The presence of red in the crown of females may make it difficult for inexperienced observers to differentiate them from young males in red–cap plumage. However, the red crown feathers of females can be distinguished from those of males by one or more of the following characteristics (Fig. 3). First, the red feathers of females often had a tawny or rusty appearance, a feature

never observed in males. Second, the red color was often present on only some of the barbs of each feather in females, while the remainder of the feather remained green, thereby creating a slightly streaked appearance (Fig. 3A). By contrast, the red feathers of young males usually had red distal barbs, and red, orange, or yellow central and proximal barbs, regardless of the number of red feathers on the crown (Fig. 3B). Third, the red feathers of females were usually the same length as the other (green) feathers on the crown, whereas the red feathers of males were usually longer than the green feathers and tended to increase in length with age (Fig. 3; S. M. Doucet, pers. obs.). Fourth, the distribution of red feathers on the crown differed between females and males. In females, red feathers could be found in the front, center, or rear of the crown. By contrast, among males in red–cap plumage, the red feathers tended to grow on the outer edges of the crown, often resulting in a split, rather than contiguous, red crown (Fig. 3A).

The development of tawny coloration in the crown of females appeared to be associated with age. Of the females we were able to age as second–year or after–second year (on the basis of plumage and mouth lining color), none of 43 second–year females had any trace of red or tawny on the crown, while 91 of 189 after–second–year females had traces of tawny or red (Fisher's Exact Test, $P < 0.00001$). Of the females that exhibited changes in plumage color during the course of the study, nine were observed or recaptured sufficiently frequently to estimate the minimum amount of time elapsed between when the bird was originally captured and when a change in plumage was first noted (5.1 ± 1.17 years; mean ± SD). Two of these females were first captured as second–year females – one of these developed a tawny crown in her eighth year while the other developed red crown feathers in her ninth year. Some females never developed red

or tawny crowns, including the three oldest, minimum–age females in this study. Two were recaptured in completely green plumage 10 years after initial capture. A third female, last observed 15 years after her initial capture, had no discernible red or tawny in her crown.

In rarer instances, females departed from the typical olive–green plumage in other ways. During the course of our study, we captured a few females with black lores or black forehead, cheek, or nape feathers (n = 21); with one or more partially or completely black wing or tail feathers (n = 11); or with blue or black wing or tail coverts or blue mantle feathers (n = 5). Apart from crown color, none of these patches was extensive enough to be easily seen in the field. Females with some tawny or red coloration in the crown were significantly more likely to show these additional male–like plumage tendencies (28 of 147) than females that were otherwise all green (6 of 504; Fisher's Exact test, $P < 0.00001$). Two of the females that had black or blue on the body were recaptured in subsequent years and no longer had black or blue in their plumage. By contrast, all females with a red or tawny crown that were recaptured in subsequent years retained a colored crown.

Timing of molt

Long–tailed manakins follow a complex pattern of molt that varies by age and sex. Among females, there is a bimodal distribution of proportion of birds molting over the course of the year, with an early peak of molt activity in March and a second peak in October (Fig. 2A). The molt occurring between February and April is limited to some head and body feathers, while the second peak corresponds to the complete, prebasic

molt. The early peak varies by age. Of the females examined in March and April that we were able to age (n = 81), 13 of 15 (86%) second–year females showed signs of molt, while only 25 of 66 (38%) after–second–year females showed signs of molt (Fisher's Exact Test, $P = 0.0003$). Unfortunately, our data do not allow us to determine whether this early peak corresponds to a limited prealternate molt, or whether it is an early beginning to the prebasic molt that is suspended through the reproductive period. This distinction would require confirmation that newly molted feathers were replaced in the subsequent prebasic molt (in the case of a limited pre–alternate molt) or not replaced in the subsequent molt (in the case of an early suspended prebasic molt).

The molt activity patterns of males in predefinitive plumage are rather more complex. We separated males by plumage stage, which generally corresponds to age (see Table 1 and *Male plumage sequence* above). As in females, a large proportion of red–cap (second–year) males showed signs of molt in March and April (Fig. 2B). Among these young males, there was a slight decrease in molt activity in May. By June, however, all red–cap males captured or examined showed signs of molt. Of the red–cap males showing signs of molt in March and April (n=19), 84% were molting only crown or head feathers. Two of these males (10%) were molting both head and body feathers, and two others (10%) were molting only body feathers. Many of these red–cap males were molting in additional red crown feathers and some were molting in black feathers on the face. In some cases, however, these males were simply molting in green crown or face feathers. The peak of molt in June corresponds to the complete, first–prebasic molt, as males were in a much heavier molt that often included flight feathers. Among black–face (third–year) and blue–back (fourth–year) males, we detected molt in only a small

percentage of males (13%) in March and April (Fig. 2 C, D). As with red–cap males, this early molt in black–face and blue–back males was largely restricted to crown and head regions. Black–face and blue–back males exhibited a peak of molt activity between June and August, which corresponded to their complete, third– and fourth–prebasic molts, respectively.

Males in definitive plumage exhibited the simplest yearly molt activity pattern (Fig. 2E). Very few (<5%) males showed any signs of molt from March to May. By June, more than one–third of definitive–plumaged males examined were molting, and molt activity in these males peaked in September.

DISCUSSION

By examining the plumage characteristics of 1,315 color–banded individuals in northwestern Costa Rica and 585 museum specimens, we documented age– and sex–related variation in plumage maturation and molt timing in Long–tailed Manakins. Male plumage development progressed in discrete, age–specific categories from an all–green juvenal plumage, through three distinct predefinitive plumages, to a definitive adult plumage in fifth–year and older birds. Although variation in female plumage was more subtle, some females acquired male–like plumage features as they aged. Our findings have important implications for elucidating the adaptive significance of delayed plumage maturation, and the potential signal function of intraspecific variation in plumage, in Long–tailed Manakins and other species.

Male plumages

Male Long–tailed Manakins progressed through the following plumage sequence. Hatch–year (juvenal) males were olive green throughout; second–year (red–cap) males were olive green with some red on the crown; third–year (black–face) males were olive green with a red crown and a black facial mask or hood; fourth–year (blue–back) males were a mixture of green and black with a red crown and a blue and green mantle; and fifth–year and older (definitive) males were black with a red crown patch and a blue mantle. This plumage sequence can be summarized by the following heuristic: from (1) a green–plumaged bird, (2) add red; (3) add black; (4) add blue; (5) take away green. Our conclusions are consistent with those of McDonald (1989a; 1993a) but differ from those of Foster (1987). In Foster's (1987) sequence, red–cap and black–face males are included in the same age class, whereas blue–back males are separated into two age classes. Differences in the interpretation of the red–cap stage may reflect the fact that birds in red–cap plumage occasionally have some black feathers on the face, particularly once they begin to molt in the early breeding season. Foster (1987) therefore (incorrectly) included black–face birds in the red–cap plumage stage. The discovery that second–year birds can be aged on the basis of differences in wing feathers now makes this distinction infallible. Foster's (1987) differentiation of males with blue, black and green plumage into two stages likely reflects a misinterpretation of the extreme variation in the proportions of these colors in the third–basic plumage. Nevertheless, our data from numerous plumage transitions show that the presence of blue mantle feathers and black body feathers (other than those on the head) is a robust criterion for defining the third–basic (blue–back) plumage.

Although there was considerable variation within plumage stages of male Long–tailed Manakins, there was little overlap between plumage stages. Two apparent exceptions to the sequence involved males that were first captured in green plumage and recaptured the following year in black–face plumage. These birds were captured before it was discovered that green birds could be aged on the basis of retained juvenal wing feathers, however, and it is likely that these were second–year males without a red crown patch, as opposed to juvenal–plumaged hatch–year males. Indeed, in a separate analysis, Clay (2001) identified two museum specimens as second–year males with all–green crowns. Moreover, the males in our study were captured in March and April, and would have fledged from exceptionally early nests if they were in fact hatch–year males. Two other males apparently molted from a red–cap plumage into a blue–back plumage. If these four males do, in fact, represent anomalous sequence transitions, they correspond to accelerations relative to the typical sequence. However, due to the rarity of these anomalies (at most 1.8 % of predefinitive plumage transitions), they probably occur arbitrarily rather than as a response to selective pressure on males to accelerate through the maturation process. Taken together, our findings strongly suggest that plumage coloration is a highly consistent signal of age in young males until they reach their fifth calendar year. This conclusion has two important implications. First, our data confirm that young males can be aged reliably in the field based on plumage features. Second, our data support the hypothesis that plumage variation can serve as a social signal of age in young males (McDonald 1993a).

Social signaling in males

The complex social system of Long–tailed Manakins is dependent upon the development of long–term cooperative alliances between males (Foster 1977; McDonald 1989a; McDonald 1989b; McDonald 1993a). Males must spend several years working their way up the dominance hierarchy to eventually to have the opportunity to display for females, and, in a minority of males, to copulate. The social stability of these mating queues appears to be strictly enforced by female mate choice, as females will often leave the dance perch at the first sign of disruption by males other than the dominant males (Foster 1987; McDonald 1989a; McDonald 1993a). The evolution of predefinitive male plumages is beneficial to both subordinate and dominant males in this type of social system. For dominant males, the predefinitive plumage of young males immediately identifies them as not posing a threat to their reproductive success (McDonald 1989b; McDonald and Potts 1994), which likely reduces the amount of aggression that young males will receive from older males. Indeed, in the congeneric Blue Manakin (*Chiroxiphia caudata*), the amount and intensity of aggression shown by dominant males toward subordinate individuals decrease with decreasing age of the target individual (Foster 1987). Moreover, the evolution of age–specific plumages can provide additional information about the status of males within the dominance hierarchy. In support of this hypothesis, a taxidermy mount experiment revealed that male Long–tailed Manakins responded more strongly to males in definitive plumage than to males in predefinitive plumage (McDonald 1993a). Moreover, responses were often initiated by non–alpha males, suggesting that the model intruders posed a threat to established male–male alliances rather than a risk of stolen copulations (McDonald 1993a). These observations

are paralleled in Blue Manakins, where aggression directed toward a transgressor was often initiated by males of intermediate rank, and directed toward the individuals nearest them in the hierarchy (Foster 1981). These studies suggest that males are most likely to challenge males of similar rank in a hierarchy and, presumably, against whom they have the greatest probability of success. The obverse of this is that males are also most likely to have to defend their own positions against challenges from members of their own or adjoining age cohorts. A model presentation experiment in another species with a graded, multi–year delay in plumage maturation, the Hawaiian 'Elepaio (*Chasiempis sandwichensis*), yielded similar findings (VanderWerf and Freed 2003).

Female plumage

Extreme sexual dichromatism is characteristic of the family Pipridae. Male manakins are usually brightly colored, while females are typically olive green (Prum 1997; Doucet et al. 2006). In this study, we document considerable variation in the plumage of female Long–tailed Manakins. Some females developed variable amounts of red or tawny coloration in the crown, reminiscent of the red crowns of males, and a small proportion of females developed black feathers on the face, head, wing, or tail. Older females were more likely to develop red or tawny crowns than were young females, and, on average, more than five years elapsed between initial female captures and the appearance of red in their crowns. Female ornamentation may have evolved as a by–product of genetic correlations between male and female traits (Lande 1987; Amundsen 2000). Strong selection for elaborate ornaments in males, combined with weaker selection against these traits in females, could lead to the expression of

ornamental coloration in females, even if it is maladaptive. Because females are solely responsible for parental care in manakins, they should experience strong natural selection for crypsis (Martin and Badyaev 1996), particularly since rates of nest predation are so high in the tropics (Stutchbury and Morton 2001). Yet bright female coloration has been reported in a number of manakin genera (Graves 1981), and, in Long–tailed Manakins, females with red crowns were more likely to express other male–like plumage characteristics. Our documented association between ornamental color and age in older females could be proximately mediated by age–related hormonal changes (Kimball and Ligon 1999). In some cases, male–like plumage characteristics other than crown color disappeared after the following molt, suggesting that they may have resulted from adventitious feather loss and replacement at a time of year when environmental cues, such as photoperiod or daytime light intensity (Gwinner 2003), led to the growth of more male–like plumage.

A less likely possibility is that the development of red crown coloration in older females serves a social signaling function. Because of its association with age, red crown coloration could signal a female's longevity or viability. Such a signal might be useful to males, who could optimize their display intensity or sperm allocation by investing more in females they deem to be of high quality (e.g., Amundsen 2000; Werner and Lotem 2003), a pattern which may explain some of the variation in male display intensity in this species. Alternatively, a signal of female age might be useful to young females, whose mate choice decisions could be influenced by those of older, experienced females when multiple females observe displaying males together (Dugatkin and Godin 1993; Doucet et al. 2004). Comparative analyses of the relationship between male and female

ornamentation, in combination with intraspecific empirical investigations of the proximate control and signal function of female traits, will help to identify the relative merit of these hypotheses in explaining the evolution of bright female plumage in manakins.

Timing of molt

Long–tailed manakins undergo a post–juvenal molt within four months of hatching and subsequently undergo a prebasic molt each year. The first–prebasic molt is incomplete, as birds of both sexes retain their juvenal remiges and distal greater coverts. Second–year birds also retain the yellow–orange mouth color typical of nestlings of this species (Foster 1976). A similar pattern of delayed maturation in mouth color has been reported in one species of bowerbird (Frith and Frith 2004). Given the considerable number of species that show delayed maturation in iris and soft–part color (Lawton and Lawton 1986), delayed maturation in mouth color may be relatively widespread and should be investigated in other species.

We found significant age– and sex–based variation in timing of molt. Females exhibited an early peak of molt activity in February and March. Second–year females were more likely to show signs of early molt than older females. Nevertheless, fewer than half of females showed signs of early molt activity, and this molt was limited to some body and head feathers. Molt activity was low from May through July in females, and increased in August to a second peak, corresponding to the complete prebasic molt, in October. The decrease in molt activity from April to July may reflect evolutionary pressure to avoid the costs of molt–breeding overlap (Foster 1974), as this period

corresponds to the peak of female nesting activity (Foster 1976). Molt and breeding are both energetically demanding (e.g., Murphy and King 1992; Lindstrom et al. 1993). As such, physiological and ecological trade–offs are expected to occur between these two life–history traits (Foster 1975b). Theory therefore predicts that molt and breeding should be temporally segregated, yet molt–breeding overlap has been reported in a number of tropical and temperate passerines (Foster 1975a; Pyle 1997) and, as we have shown here, occurs at the population level in Long–tailed Manakins (Fig. 2). However, our data show that in May, June, and July, fewer than 15% of females exhibited any signs of molt. Later in the season, females' primary resource consuming activity can switch from breeding to molt (see Foster 1975a), which may explain the second, most prominent peak of female molt activity in October.

Though they provide no parental care, male Long–tailed Manakins may expend considerable amounts of energy in breeding–related activities and are**,** therefore**,** also expected to avoid molt–breeding overlap. Dominant (alpha and beta) males perform energetically demanding dance displays for females and sing duets at high rates to attract females to their leks (up to 5000 vocalizations/day, Foster 1977; McDonald 1989b; Trainer and McDonald 1993). By June, there is usually a notable decrease in male display activity, especially at the least successful leks (S. M. Doucet and D. B. McDonald, unpubl. data), which, as we have shown here, corresponds to a sharp increase in proportion of males in definitive plumage showing signs of molt. Females, on the other hand, are known to nest until at least July, and probably until September (Foster 1976). Male display activity is intrinsically linked to the availability of receptive females. A successful copulation, however, marks only the beginning of a female's parental

responsibilities. Thus, the reproductive periods of dominant males and females are inherently staggered, which may explain why the post–breeding molt of males in definitive plumage peaks earlier than that of females. Moreover, many definitive–plumaged males occupy subordinate ranks at leks. These males are expected to expend much less energy on display than dominant males, and to curtail their breeding season activities sooner**.**

Over the course of the breeding season, young male Long–tailed Manakins in predefinitive plumage form affiliations with other males at particular lek sites, often practicing courtship displays on dance perches used by dominant males. Young males are tolerated at dance perches only in the absence of females, however, as only dominant males display for, and copulate with, females (McDonald 1989a). Moreover, young males are resighted at leks less frequently than older males (McDonald 1989a), suggesting that they invest less time and energy in breeding–season activities than do older males. Thus, young males can begin molting earlier in the season than older individuals. Correspondingly, blue–back and black–face males exhibit an earlier peak of molt activity than males in definitive plumage.

Early molt in red–cap males is more difficult to interpret. Many males in red–cap plumage begin to molt in March, at the onset of the breeding season. Moreover, this molt is apparently restricted to the crown and head. Indeed, these males often molt in additional red crown feathers and black face feathers, thereby accelerating their transition into black–face plumage. Dominance interactions among males of the same age cohort may explain this early molt. If crown and mask color allow males to assert their dominance over members of their own age cohort, then selection might favor the

acceleration of such a molt. Males in predefinitive plumages, particularly younger ones, are subject to opposing selective forces: by signaling their lesser age, they may reduce the aggression directed toward them by older males; at the same time, however, it might benefit them to develop, as much as possible, the traits that signal their prowess to males of the same cohort (Foster 1987; McDonald 1993a). This conflict may explain why some species progress through several distinct predefinitive plumage stages rather than molting into the same predefinitive plumage for several years, as has been shown in a number of other species (Frith and Beehler 1998; Frith and Frith 2004).

Implications

Male Long–tailed Manakins in predefinitive plumage have testes capable of producing sperm (Foster 1987). Thus, delayed plumage maturation in this species represents an instance of heterochrony, and, more specifically, of neoteny; that is, a slowing down of somatic maturation relative to sexual maturation (Lawton and Lawton 1986). Mean testis size among 60 definitive plumaged males was 29.3 ± 16.16 mm^3 (Foster 1987). Interestingly, the testis size of a beta male, known to have been in definitive plumage for several years, was 75 mm^3, nearly three standard deviations above the mean (D. B. McDonald, unpubl. data), suggesting that plumage maturation likely precedes full testis maturation in this species. Long–tailed manakins may, therefore, exhibit both neoteny and progenesis (where somatic maturation precedes reproductive maturation). Lawton and Lawton (1986) proposed that heterochronic trends in plumage, iris, and soft–part maturation are associated with complex social organization within the Corvidae and may have played a role in speciation among closely related corvids. As

empirical investigations of delayed plumage maturation become more widely available, particularly among tropical species, it will be possible to examine patterns of heterochrony, and the complex mixing of neotenic and progenetic features, in other groups that vary in degree of social organization, including other manakins (Prum 1997). The increasing availability of well–supported phylogenies will greatly facilitate these comparative investigations. Studies of delayed plumage maturation across a wider variety of taxa, particularly species that differ from the typical north–temperate pattern, are likely to reveal that the adaptive significance of delayed plumage maturation cannot be encompassed by a single hypothesis. Moreover, accumulating evidence of interspecific variation in both patterns and consequences of delayed plumage maturation suggest broader evolutionary implications than originally anticipated.

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Table 1. Summary of plumage development in male long–tailed manakins. Plumage descriptions summarize the typical appearance of males in each plumage stage.

Age range (months since hatching)	Age name [a] (calendar year)	Plumage stage	Name of plumage stage	Plumage description
0–4	hatch–year	juvenal	juvenal	Olive green throughout with paler wash below
4–15	second–year	first–basic	red–cap	Olive green body and flight feathers; partial red crown
15–27	third–year	second–basic	black–face	Olive green body and flight feathers; small red crown; black face or hood
27–39	fourth–year	third–basic	blue–back	Black and green body and flight feathers; red crown; blue and green mantle
≥ 39	≥ fifth–year	definitive–basic	definitive	Black body and flight feathers; red crown; blue mantle

[a]Age terminology follows (Pyle 1997); see Methods for explanation.

Table 2. Summary of sequential plumage transitions recorded in male long–tailed manakins.

Plumage transitions	Number of recorded instances
Typical	
Green to red-cap	1
Red–cap to black–face	60
Black–face to blue–back	60
Blue–back to definitive	48
Definitive to definitive	343
Potential anomalies	
Green to black–face	2
Red–cap to blue–back	2

FIGURE CAPTIONS

Figure 1. Photographs showing typical appearance of long–tailed manakins in different plumage stages. (A) female or male in juvenal plumage, (B) male in red–cap plumage, (C) male in black–face plumage, (D) male in blue–back plumage, (E) male in definitive plumage. Photo in (E) by Richard Laval.

Figure 2. Monthly proportions of long–tailed manakins showing signs of molt at time of capture for birds in different plumage stages. Data are from mist–net captures in northwestern Costa Rica (n = 867) and museum specimens (n = 585). Data are pooled across years and numbers above bars indicate the total number of birds captured in each month. Although plumage stages are indicated on each graph, letters are included to facilitate referencing in the text and comparisons with Figure 1.

Figure 3. Photographs showing the crowns of: (A) a young male long–tailed manakin in red–cap plumage and (B) a female long–tailed manakin with some red crown feathers. Note differences in the distribution of red on the crown, the distribution of red on individual feathers, and the length of red crown feathers.

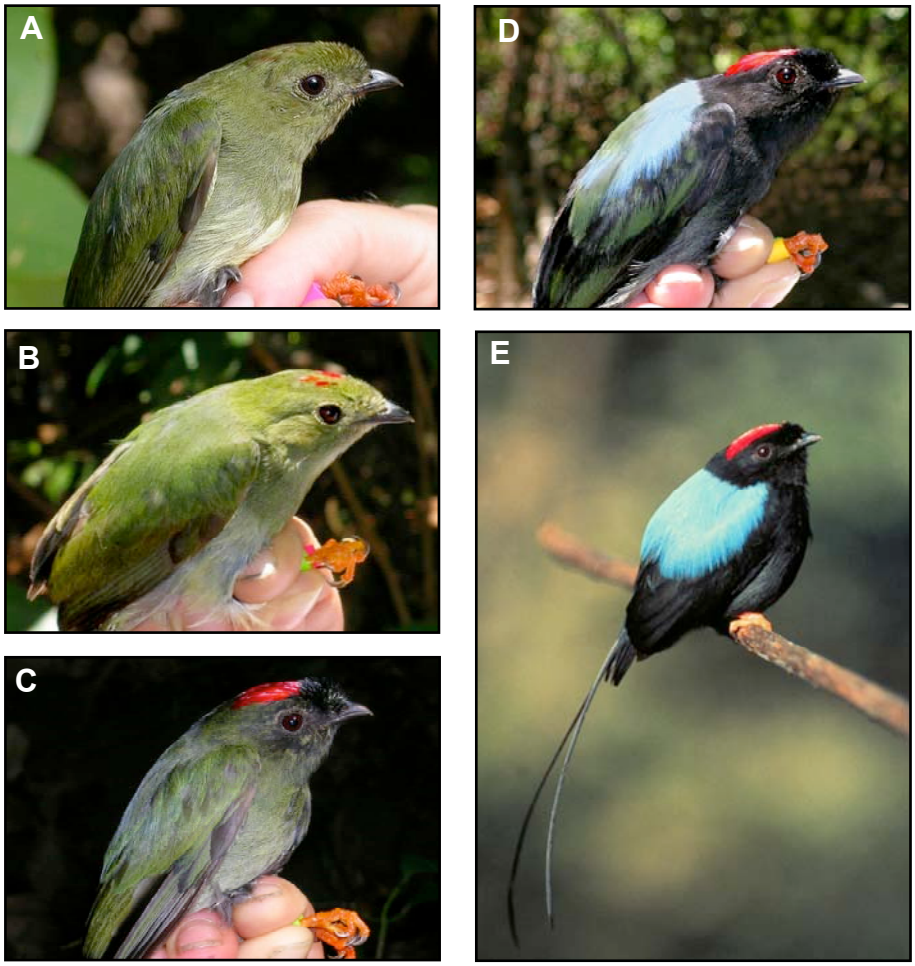

Figure 1.

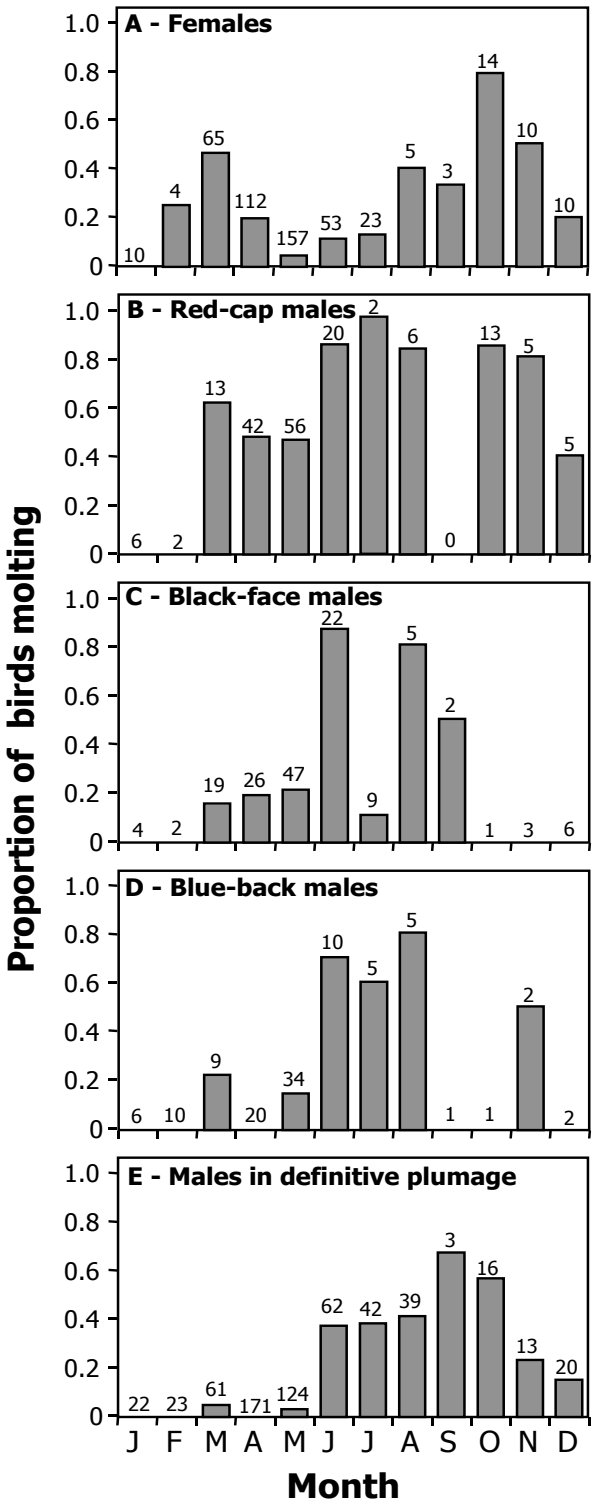
A - Females
10
4
65
112
157
53
23
5
3
14
10
10
B - Red-cap males
6
2
13
42
56
20
2
6
0
13
5
5
C - Black-face males
4
2
19
26
47
22
9
5
2
1
3
6
D - Blue-back males
6
10
9
20
34
10
5
5
1
1
2
2
E - Males in definitive plumage
22
23
61
171
124
62
42
39
3
16
13
20
1.0
0.8
0.6
0.4
0.2
0
J F M A M J J A S O N D
Month
Proportion of birds molting

Figure 2.

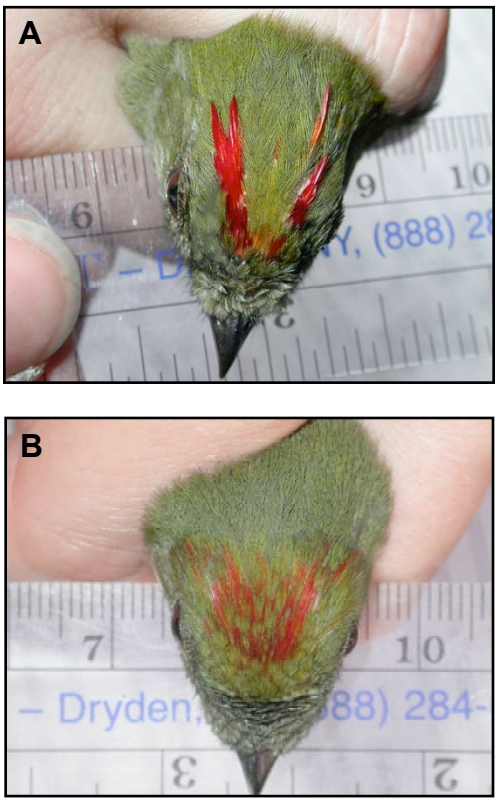

Figure 3

CHAPTER 2. COMPLEX PATTERNS OF SEXUAL DIMORPHISM IN *CHIROXPHIA* MANAKINS: INTERACTING EFFECTS OF NATURAL AND SEXUAL SELECTION?

ABSTRACT

Sexual size dimorphism is a widespread biological phenomenon. In birds, males are most often the larger sex, although there exists extensive variation in both the direction and magnitude of dimorphism across species. Several hypotheses have been proposed to explain the evolution of sexual size dimorphism, and these can be classified into two categories: ecological hypotheses and sexual selection hypothesis. In this study, we investigate the explanatory power of these hypotheses in elucidating patterns of sexual size dimorphism in *Chiroxiphia* manakins, with particular emphasis on the long–tailed manakin, *C. linearis*. We captured 361 wild long–tailed manakins and measured variation in six morphological traits: wing size and shape, body mass, bill size and shape, the length of elongated central rectrices (tail plumes), tail length, and tarsus length. We measured a subset of these traits in 872 museum specimens distributed among all five species in the genus *Chiroxiphia*. Male long–tailed manakins has longer, broader wing that bore less weight per unit area than females; they also had longer tarsi and tail plumes than females. Females weighed more, had longer, wider bills and longer tails than males. Patterns of sexual dimorphism in bill length, tarsus length, and body mass were generally similar among other *Chiroxiphia* manakins. Our findings were consistent with the predictions of three hypotheses. First, compared with females, the body mass and wing shape of males should enhance flight maneuverability during courtship displays, as predicted by the Acrobatic Displays Hypothesis. Second, males and females diverged in bill size and shape in a direction opposite to that predicted from differences in tarsus length, as predicted by the Intersexual Competition Hypothesis. Finally, the higher body mass, higher wing loading, higher wing aspect ratio, and larger bills of females should

enhance their capacity to catch flying insects, carry more weight, lay larger eggs, and forage more efficiently, as predicted by the Reproductive Role Division Hypothesis. Our findings suggest that both natural and sexual selection have had an important influence on the evolution of complex patterns of sexual dimorphism in *Chiroxphia* manakins, although further research is needed to confirm the relative influence of each selection mechanism on particular morphological traits within this group. Our study identifies a promising approach for comparative investigations of sexual dimorphism in other avian groups exhibiting similar life–history traits.

INTRODUCTION

Systematic differences in body size between the sexes of a species is a widespread biological phenomenon (Darwin 1871; Ralls 1976; 1989; Shine 1989; Andersson 1994a; Fairbairn 1997). Among invertebrates and poikilothermic vertebrates, females are most often the larger sex, whereas in mammals and birds, males are usually the larger sex (Shine 1989; Andersson 1994a; Fairbairn 1997). Ever since the publication of Darwin's treatise on sexual selection (Darwin 1871), such sexual dimorphism has been of broad interest to evolutionary biologists, and several hypotheses have been proposed to explain the evolution and maintenance of size–dimorphic traits. These hypotheses can be classified into two categories: 'Sexual Selection Hypotheses' and 'Ecological Hypotheses'. It is important to recognize that although some of these hypotheses focus on the selective advantage of a particular body size in one sex, the evolution of sexual size dimorphism ultimately results from sex–specific patterns of selection on body size. Differences in the sum of all natural and sexual selection pressures can lead to the evolution of different size optima for different traits in males and females (Price 1984; Hedrick and Temeles 1989; Andersson 1994a; Badyaev et al. 2000). Because of strong genetic correlations between the sexes (Lande 1980), dimorphism evolves only when selection for a particular trait in one sex is counterbalanced by opposing selection in the other sex. Thus, depending on the strength and direction of selection, the sum of selection pressures in males and females can either reinforce or constrain the evolution of dimorphism (Price 1984; Schluter et al. 1991; Merilä et al. 1997; Badyaev et al. 2000; Badyaev 2002).

According to sexual selection hypotheses, size–dimorphic traits evolve because they directly (via female choice) or indirectly (via male–male competition) enhance mating opportunities (Darwin 1871; Andersson 1994a). In particular, the 'Direct Competition Hypothesis' proposes that male–biased size dimorphism evolves because larger body size in males is favored in competitions over mates or resources (Table 1, Darwin 1871; Andersson 1994a). In elephant seals, *Mirounga angustirostris*, for example, males engage in physical contests over access to reproductive females; larger males are more successful in these contests and experience greater reproductive success (Le Boeuf and Reiter 1988). Indeed, in many species, larger males are more successful than smaller males at monopolizing access to females, high–quality territories, or other resources (Andersson 1994a).

Sexual selection can also favor the evolution of the reverse pattern of size dimorphism, with females being larger than males. Occasionally, reversed sexual dimorphism accompanies a near complete reversal of sex roles, where females become the competitive sex. This pattern, also consistent with the Direct Competition Hypothesis, is well–documented in avian groups such as jacanas and coucals (e.g., Andersson 1995; Emlen and Wrege 2004; Goymann et al. 2004; Andersson 2005) as well as in sex–role reversed seahorses and pipefish (Jones and Avise 2001). Alternatively, in birds, small male body size may be favored by sexual selection in species wherein males perform acrobatic aerial sexual displays (Payne 1984; Jehl and Murray 1986; Hedenström and Møller 1992; Blomqvist et al. 1997). This 'Acrobatic Displays Hypothesis' proposes that small body size enhances a male's ability to perform acrobatic displays requiring a high degree of agility and coordination (Table 1). Comparative studies suggest that sexual

selection for male display agility may be responsible for the evolution of small male body size in shorebirds and waders (e.g., Figuerola 1999; Szekely et al. 2000).

Two primary ecological hypotheses have been proposed to explain the evolution of sexual size dimorphism: the 'Intersexual Competition Hypothesis' and the 'Reproductive Role Division Hypothesis' (Table 1). The Intersexual Competition Hypothesis proposes that sexual size dimorphism evolves to reduce intersexual competition for food resources (Darwin 1871; Selander 1966; Selander 1972; Hedrick and Temeles 1989; Shine 1989). The reasoning is that sexual dimorphism in body size, particularly for feeding–related traits, allows the sexes to exploit different ecological niches and thereby reduces intersexual competition for food (Darwin 1871; Selander 1966; Selander 1972; Hedrick and Temeles 1989; Shine 1989). Testing this hypothesis poses several difficult challenges, and conclusive supporting evidence remains relatively sparse (Hedrick and Temeles 1989; Shine 1989). Striking examples exist nonetheless, including mosquitoes, where female mouth parts are adapted for sucking blood whereas male mouthparts are adapted for ingesting nectar (Darwin 1871; Proctor et al. 1996), and the extinct New Zealand huia, *Neomorpha acutirostris*, where remarkable sexual differences in bill size and shape presumably allowed males and females to forage on different prey types (Darwin 1871; Andersson 1994a). Recently, Temeles and colleagues (Temeles et al. 2000; Temeles and Kress 2003) provided compelling evidence that pronounced bill length dimorphism in the purple–throated carib hummingbird, *Eulampis jugularis*, is associated with specialization in each sex for feeding on a different species of *Heliconia*.

A second ecological hypothesis, the 'Reproductive Role Division' hypothesis (Table 1), proposes that sexual dimorphism evolves as a consequence of the different reproductive roles of males and females (Darwin 1871; Ralls 1976; Hedrick and Temeles 1989; Shine 1989; Andersson 1994a; Fairbairn 1997). In many animals, the size of gametes differs between males and females (anisogamy) and the sexes invest differentially in various aspects of reproduction (e.g., incubation, brooding, nest defense, offspring provisioning). Such differences may lead to the evolution of different optimal body sizes for males and females. In vespertilionid bats, for example, large female body size may have evolved to facilitate the female's task of carrying young offspring (Myers 1978). Selection based on reproductive role division can result in either male–biased size dimorphism or female–biased size dimorphism (Andersson 1994a), and the outcome will largely depend on the degree of anisogamy, the mating system, and the division of parental care.

Ecological and sexual selection hypotheses are commonly presented as alternative explanations for the evolution of sexual size dimorphism and are often studied independently (Hedrick and Temeles 1989). As we have emphasized, however, natural and sexual selection may interact to lead to the evolution of different size optima in males and females (Price 1984; Hedrick and Temeles 1989; Andersson 1994a; Badyaev et al. 2000). Thus, studies that adopt an integrative approach considering both ecological and natural hypotheses should provide a clearer picture of the selective forces shaping sexual dimorphism.

In this study, we evaluate the explanatory power of the Direct Competition, Acrobatic Displays, Intersexual Competition and Reproductive Role Division hypotheses

in elucidating patterns of sexual size dimorphism in *Chiroxphia* manakins, with particular emphasis on the long–tailed manakin, *C. linearis*. Manakins (Aves: Pipridae) are small, largely frugivorous, suboscine passerines distributed throughout the Neotropics. Most manakins follow a lek–based mating system wherein assemblages of displaying males compete for copulations with females (Snow 2004). Males typically perform complex, acrobatic courtship displays to attract females (Prum 1994; Prum 1997; Snow 2004). This mating system leads to extreme female choosiness and high variance in male mating success (McDonald 1989b; McDonald 1993; Shorey 2002; Snow 2004). Consequently, the intensity of sexual selection is thought to be particularly high among manakins in general (Prum 1997; Snow 2004), and *Chiroxiphia* manakins in particular (Foster 1981; McDonald 1993). Females, on the other hand, do not appear to compete for copulations (Snow 2004). Moreover, as in all lek–mating species (Höglund and Alatalo 1995), female manakins are entirely responsible for all aspects of parental care, from building the nest, to incubating the eggs, to caring for nestlings and fledglings (Snow 2004). The sexes also differ in the sizes of their home ranges, with females and young males having home ranges several times as large as those of adult males (Graves et al. 1983; McDonald 1989a; McDonald 1989b; Théry 1992; Snow 2004). And, in long–tailed manakins at least, the sexes exhibit a remarkable divergence in demographic variables, including age at first reproduction, age–specific fecundities, and age–specific survival probabilities (McDonald 1993). Together, these observations suggest that the selection pressures shaping morphology may be remarkably divergent in male and female manakins.

Chiroxiphia manakins differ from other manakins in two important ways. First, they exhibit extended delays in plumage maturation (Foster 1987a; Snow 2004; DuVal

2005), with long–tailed manakins, *C. linearis* exhibiting the longest recorded delay of four years (Doucet et al. 2006). This delayed plumage maturation parallels a delay in behavioral maturation, as immature males usually do not compete for copulations with females (e.g., Foster 1981; McDonald 1989a; McDonald 1989b). The morphology of young males may therefore be particularly informative, as it should reflect an increasing influence of sexual selection with age. Second, males cooperate to perform displays for females (Snow 2004). Although manakins in other genera also perform joint displays, obligate cooperative display is apparently restricted to *Chiroxiphia* (Snow 2004). In long–tailed manakins, males establish age–structured dominance hierarchies at leks. The two most dominant males at each lek perform complex, dual–male displays for females (Foster 1977; McDonald 1989a; McDonald 1989b). These displays involve alternating backward leapfrog displays, where each male alternately jumps over the other male while hovering briefly at the apex of the jump, and butterfly displays, where males alternately or simultaneously perform slow, labored butterfly flights and rapid dives between the display perch and branches and vines up to 20 m away (McDonald 1989b). Displays in other *Chiroxiphia* manakins are similar (Snow 2004), although in *C. caudata* they frequently involve more than two males (Foster 1981).

Predictions: Sexual Selection Hypotheses

We investigated sexual dimorphism and ontogenetic variation in six morphological traits of long–tailed manakins (Table 1): (1) wing size and shape; (2) body mass; (3) bill size and shape; (4) tail plume length; (5) tail length; and (6) tarsus length. We also measured a subset of these traits among museum specimens of all five species in

the genus *Chiroxiphia*. According to the Direct Competition Hypothesis, male–male competition should favor larger overall body size in males (Table 1) (Darwin 1871; Andersson 1994a). According to the Acrobatic Displays Hypothesis, selection should favor male morphology that enhances the efficiency of male displays (Table 1) (Jehl and Murray 1986). Based on flight aerodynamic theory, selection for flight endurance as well as maneuvering and hovering and ability should favor a combination of low body mass and long, broad wings among adult males (details in methods) (Jehl and Murray 1986; Rayner 1988; Pennycuick 1989; Norberg 1990; Hedenström and Møller 1992). In addition, longer tails should be favored to improve lift and maneuverability whereas shorter tail plumes (elongated central rectrices) should be favored to decrease drag (Balmford et al. 1993; Thomas 1993; Norberg 1995).

Predictions: Ecological Hypotheses

According to the Intersexual Competition Hypothesis, the sexes should exhibit morphological divergence in traits relating to feeding, although the direction of dimorphism cannot be predicted (Selander 1966; Selander 1972; Hedrick and Temeles 1989; Shine 1989). Manakins are gape–size limited frugivores; that is, they swallow most fruits whole (Pratt and Stiles 1985; Wheelwright 1985; Foster 1987b; Snow 2004). This method of feeding imposes constraints on both the maximum size of fruits that can be eaten and the optimal size for minimizing handling time (Wheelwright 1985; Foster 1987b; Levey 1987). This hypothesis therefore predicts that males and females should particularly diverge in bill size and shape. According to the Reproductive Role Division Hypothesis, divergence in the reproductive roles of male and female manakins should

result in morphological divergence between the sexes (Darwin 1871; Ralls 1976; Shine 1989). Both male–biased and female–biased size dimorphism can evolve through this mechanism, although most hypotheses ascribed to this category predict that large body size should be favored in females (Darwin 1871; Ralls 1976; Shine 1989; Andersson 1994a). Larger females may benefit from increased fecundity, improved nest defense, and a greater energy storage capacity (Andersson 1994a). Reproductive Role Division Hypotheses predicting smaller body size in females (e.g., rapid onset of breeding in fluctuating environments or early maturation) (Andersson 1994a) do not apply to the breeding system of manakins. In addition to larger female body mass, flight aerodynamic theory predicts that Reproductive Role Division should favor the evolution of long, narrow wings in females, which would provide greater load–lifting capacity and higher potential flight speeds during foraging flights (details in methods) (Jehl and Murray 1986; Rayner 1988; Pennycuick 1989; Norberg 1990; Hedenström and Møller 1992). Because manakins are gape–size limited frugivores (Wheelwright 1985; Foster 1987b) and females are solely responsible for feeding offspring, large, wide bills may also be favored in females. Gape size in frugivores is positively correlated with the mean and maximum (but not minimum) size of fruits eaten (Pratt and Stiles 1985; Wheelwright 1985) and in long–tailed manakins, there is a close correspondence between gape size and both the size distribution of fruit species eaten and the size distribution of individual fruits eaten (Wheelwright 1985). Larger bills would therefore allow females to access a broader range of fruit species and fruit sizes, as might be favored by the demands of uniparental care.

Predictions: predefinitive males

To have any chance of reproducing, male long–tailed manakins must first survive for at least eight years (McDonald 1993). Thus, early male ontogeny should favor traits that enhance survival. As they age, however, young males should face increasingly intense selection to adopt the morphology most beneficial to survival and reproduction among older males (Badyaev 2002). If female morphology more closely approaches optimal body size, and sexual selection drives older males away from this optimum (Price 1984), we predict that young males should exhibit intermediate trait morphology between females and older males.

METHODS

Wild birds

We studied long–tailed manakins from March to July, 2003–2005 in Santa Rosa National Park, Guanacaste, Costa Rica (10˚ 40’ N, 85˚ 30 W). We captured 361 long–tailed manakins using mist nets. We fitted each individual with a unique combination of up to three colored leg bands and one numbered aluminum leg band. Females are usually entirely olive–green, whereas males show age–specific variation in plumage coloration (Doucet et al. 2006). We aged male birds in predefinitive plumage as second–year, third–year, or fourth–year males based on plumage characters as described in Doucet et al. (2006). Males in definitive adult plumage, hereafter adult males, were assumed to be in their fifth calendar year or older (Doucet et al. 2006). Genetic sexing of all birds captured in 2003 (n = 145) using sex–specific genetic primers (Griffiths et al. 1998) confirmed that these criteria are sufficient to accurately sex birds during the breeding

season. Additionally, we confirmed the sex and/or age assignments of many individuals by the presence of a brood patch, sex–specific behavior exhibited during behavioral observations at leks, as well as stasis (females) or changes (males in predefinitive plumage) in plumage coloration in subsequent years (Doucet et al. 2006).

Morphometry

We measured the right tarsus length and bill length (from the anterior edge of the nare to the tip of the upper mandible) of each bird to the nearest 0.1 mm and the unflattened wing chord and tail length of each bird to the nearest 0.5 mm. Long–tailed manakins have a pair of elongated central rectrices (tail plumes). We measured the length of both the left and right tail plume of each bird and used the mean of these two measurements in our analyses. We weighed each bird to the nearest 0.25 g. The mass of females can be artificially inflated if they are carrying eggs, and indeed, females with brood patches were significantly heavier than females without brood patches ($t = –3.21$, $n = 34, 146$, $P = 0.001$). However, this pattern could be caused by any combination of follicular growth (Kullberg et al. 2002a; Guillemette and Ouellet 2005), increased energy reserves during incubation (Kullberg et al. 2002b), and/or an association between body size and the likelihood of reproducing in females. Most importantly for the purposes of this study, the sex differences in body mass reported below remain highly significant even when only females without brood patches are considered ($P < 0.0001$).

To obtain more detailed information about bill and wing shape, we collected additional measurements of individuals captured in 2005. We measured the total length of the bill from the tip of the upper mandible to the nasofrontal ridge, as well as the width

and depth of the bill at the nares (to the nearest 0.1 mm). We collected wing size and shape measurements following the methods outlined by Pennycuick (1989) and Hertel and Ballance (1999). We measured total wing length as the distance from the sternum to the tip of the outstretched wing along the ventral surface of the bird; wingspan was thus twice the total wing length (Fig. 1). To calculate wing area, we traced the fully outstretched wing with the wing positioned ventral side down. We digitized this tracing and used the program Image J 1.3 (National Institutes of Health, USA) to determine its area. The tracing was shorter than the wing length because of the body area between the proximal edge of the wing and the sternum, known as the root box (Pennycuick 1989). This rectangular area was quantified by multiplying the width of the wing where it meets the body with the difference between the total wing length and the length of the wing tracing (Fig. 1). The root box was added to the partial wing area and the total area was therefore twice this value (Fig. 1). We used these data to calculate aspect ratio and wing loading (Rayner 1988; Norberg 1990; Hertel and Ballance 1999). We calculated aspect ratio as wing span^2/wing area. Higher values for this index indicate a comparatively narrow wing and lower values a comparatively broad wing. We calculated wing loading as body mass X gravitational acceleration constant (a)/ wing area. Wing loading describes the weight (force) that is borne per unit area of the wings in Newtons/m^2.

We used aerodynamic theory to evaluate how variation in these traits would influence flight performance in manakins (Andersson and Norberg 1981; Rayner 1988; Pennycuick 1989; Norberg 1990). We focused on 10 flight parameters as outlined in Hedenström and Møller (1992)(Table 2). (1) Minimum turning radius and (2) roll acceleration influence the ability to make tight turns and are therefore measures of

maneuverability. (3) Minimum gliding speed influences the ability to make accurate landings. (4) Minimum sink speed in gliding flight, (5) mimimum power in hovering flight, and (6) power in flapping flight all influence endurance. (7) Best glide ratio is proportional to the maximum lift–to–drag ratio and is associated with generally efficient flight performance. (8) Terminal speed in gliding dive and (9) maximum linear acceleration are speed parameters usually thought to influence hunting capacity. (10) Climb rate in flapping flight influences load–lifting capacity (Hedenström and Møller 1992). We followed the predictions outlined by Hedenström and Møller (1992) to determine how variation in body mass, wing span, wing area, aspect ratio, and wing loading would influence these different flight parameters and determined which sex would be expected to show better performance for each parameter based on phenotypic differences.

Museum data

We measured specimens from each of the five species in the genus *Chiroxiphia*, totaling 872 specimens, at the Louisiana State University Museum of Natural Science and the American Museum of Natural History (Table 3). We measured the tarsus length and bill length of each specimen as described above for wild birds. We noted the mass of birds from specimen tags when available. Birds in entirely green plumage were identified as males or females as indicated on specimen tags. Birds of unknown sex were excluded from our analyses.

Statistical analyses

We used Shapiro–Wilk tests to determine whether variables were normally distributed and used non–parametric tests when variables departed significantly from normality. Small variations in sample size occur because not all information was available for all birds. We did not measure traits that appeared anomalous (e.g., bill deformities, broken feathers, growing feathers).

RESULTS

Ontogeny of male morphology

There were significant differences in wing length (ANOVA, $F = 10.64$, $df = 3, 171$, $P < 0.0001$), tail length (ANOVA, $F = 37.31$, $df = 3, 168$, $P < 0.0001$), tail plume length (ANOVA, $F = 227.46$, $df = 3, 164$, $P < 0.0001$), and mass (ANOVA, $F = 8.43$, $df = 3, 169$, $P < 0.0001$) among male long–tailed manakins of different age classes (Fig. 2A–D). Tail length ($r_s = –0.66$, $n = 172$, $P < 0.0001$) and mass ($r_s = –0.22$, $n = 173$, $P = 0.003$) were significantly negatively correlated with age class, whereas tail plume length was significantly positively correlated with age class ($r_s = 0.85$, $n = 168$, $P < 0.0001$). Wing length was also significantly positively correlated with age class ($r_s = 0.24$, $n = 172$, $P < 0.0001$), but this pattern was driven by the significantly shorter wings of second–year males (Tukey–Kramer test, $P < 0.05$). By contrast, there were no significant differences in bill length (ANOVA, $F = 1.80$, $df = 3, 168$, $P = 0.15$) or tarsus length (ANOVA, $F = 0.95$, $df = 3, 165$, $P = 0.41$) among males of different age classes (Fig. 2E, F). Thus, to maximize our sample sizes in comparisons with females, we pooled males of all age classes for analyses of bill and tarsus length, pooled after–second–year males for analyses

of wing length, and included only males in definitive plumage for analyses of the remaining morphological traits.

Sexual dimorphism in long–tailed manakins

Male long–tailed manakins had significantly longer wings (Fig. 3A; $t = –8.97$, $n = 298$, $P < 0.0001$), tail plumes (Fig. 3C; $t = –59.40$, $n = 143, 59$, $P < 0.0001$), and tarsi (Fig. 3F; $t = –15.17$, $n = 346$, $P < 0.0001$) than females. By contrast, females had significantly longer tails (Fig. 3B; $t = 6.73$, $n = 238$, $P < 0.0001$) and bills (Fig. 3E; $t = 12.71$, $n = 175, 170$, $P < 0.0001$), and weighed significantly more (Fig. 3D; $t = 11.58$, $n = 242$, $P < 0.0001$), than males.

Sex differences in individual traits can result from overall differences in body size. We verified that sex differences in morphological traits were not simply an allometric consequence overall differences in body size by regressing Log_{10}–transformed morphological traits on Log_{10} of tarsus length and comparing the residuals of these regressions between males and females. We used tarsus length as an index of structural body size as it is not influenced by short–term variation in nutritional condition and does not change with age (Rising and Somers 1989; Freeman and Jackson 1990; Webster 1997). Even when controlling for tarsus length, males had significantly longer tail plumes ($t = –16.16$, $n = 226$, $P < 0.0001$) and wings ($t = –2.56$, $n = 234$, $P = 0.01$) than females. Female–biased dimorphism in bill length, tail length, and mass cannot be simple by–products of differences in structural body size, as females had shorter tarsi than males. Indeed, dimorphism in these traits increases when controlling for tarsus length (all $P < 0.0001$).

Morphological predictors of sex and age classes

We used discriminant function analysis (DFA) to determine whether the six morphological traits we measured could accurately differentiate birds in different age and sex classes. We entered tarsus length, bill length, wing length, tail length, tail plume length, and mass into the DFA as measurement variables and age/sex class as classification variables. We found significant morphological differentiation according to age and sex class (Fig. 4; $F = 503.8$, $df = 6, 322$, $P < 0.0001$). The first and second canonical axes explained 90.2% and 8.9% of age– and sex–related variation in morphology, respectively, and the discriminant analysis predicted sex and/or age class with 84% accuracy. Most errors in classification involved the misclassification of females as second–year males and vice–versa (20% of errors), or the misclassification of males into an age class adjacent to the one they belonged in (72% of errors). No classification errors involved the misclassification of females as males in definitive adult plumage or vice versa.

Sexual dimorphism in bill size and shape

All three components of bill size were sexually dimorphic in long–tailed manakins. Females had significantly longer ($t = 6.6$, $n = 21, 28$, $P < 0.0001$), wider ($t = 4.0$, $n = 21, 27$, $P = 0.0002$) and deeper ($t = 3.53$, $n = 21, 28$, $P = 0.0008$) bills than males. However, both bill depth ($r = 0.47$, $n = 49$, $P = 0.0007$) and width ($r = 0.30$, $n = 49$, $P = 0.04$) were positively correlated with bill length. We therefore used the residuals of regressions of bill width and depth on bill length to investigate dimorphism in bill shape. Residual bill width ($t = 2.24$, $n = 21, 27$, $P = 0.03$) but not depth ($t = 1.03$, $n = 21, 28$, $P =$

0.30) was significantly higher in females than males, indicating that the bills of females were significantly wider than predicted from increases in length alone.

Ontogeny of male wing size and shape

There were significant differences in wing area (Fig. 5A; $F = 6.85$, $df = 3, 24$, $P = 0.002$), aspect ratio (Fig. 5B; $F = 6.76$, $df = 3, 24$, $P = 0.002$), and wing loading (Fig. 5C; $F = 3.29$, $df = 3, 24$, $P = 0.004$), but not wing span (Fig. 5D; $F = 0.82$, $df = 3, 24$, $P = 0.49$) among males in different age classes. Wing area increased significantly with age ($r_s = 0.68$, $n = 27$, $P < 0.0001$) whereas aspect ratio decreased significantly with age ($r_s = –0.65$, $n = 27$, $P = 0.0001$). Wing loading was not linearly related to age, as it was intermediate among second– and third–year males, highest among fourth–year males, and lowest among definitive plumaged males (Fig. 5C). We therefore pooled males of all age classes for analyses of sex differences in wingspan, but restricted our analyses to males in definitive plumage analyses of sexual differences in wing area, aspect ratio, and wing loading.

Sexual dimorphism in wing size and shape

Male long–tailed manakins had significantly larger wing areas (Fig. 6A; $t = –7.66$, $n = 21, 7$, $P <0.0001$) and longer wing spans (Fig. 6D; $t = –6.23$, $n = 21, 28$, $P <0.0001$) than females, whereas females had significantly higher wing loading (Fig. 6C; $t = 6.62$, $n = 21, 7$, $P <0.0001$) and aspect ratios (Fig. 6B; $t = 2.70$, $n = 21, 7$, $P = 0.01$) than males. Based on aerodynamic theory, we were able to determine how changes in body mass and these four measures of wing size and shape would influence 10 different flight

parameters (Norberg 1990; Hedenström and Møller 1992), which then allowed us to predict which sex would show better performance for each flight parameter (Table 2). Based on their morphology, males are predicted to outperform females in maneuverability, landing accuracy, flight endurance, and general flight efficiency (Table 2). By contrast, females are predicted to outperform males in load–lifting capacity and some components of flight speed (Table 2).

We used measures of wing size and shape, bill size and shape, and body mass to construct a three–dimensional morphospace, which provided a graphical representation of relationships between individuals of each sex. We used principal components analysis (PCA) to calculate a single variable that could summarize variation for each of bill morphology and wing morphology. We performed a first PCA on bill width, depth, and length. All three measures had high positive loadings on the first PC axis, which explained 54% of the variation in bill morphology. We performed a second PCA on wingspan, wing area, and aspect ratio. Wing area and wingspan had high positive loadings on the first PC axis whereas aspect ratio had a moderate negative loading on this axis, which explained 58% of the variation in wing morphology. We standardized body mass, bill PC1, and wing PC1 to a mean of zero and a standard deviation of one and plotted these values in a three–dimensional morphospace (Fig. 7). Male and female long–tailed manakins occupied substantially different regions of the morphospace with relatively little overlap. Males had a small body mass, long, broad wings, and short, narrow bills. By contrast, females had a larger body mass, short, narrow wings, and long, broad bills (Fig. 7).

Sexual dimorphism in *Chiroxiphia* manakins

Museum specimens of long–tailed manakins exhibited similar patterns of dimorphism to those we documented in wild–caught birds. Males had significantly longer tarsi ($t = –7.1$, $n = 119$, $P < 0.0001$) than females, whereas females had significantly longer bills ($t = 4.5$, $n = 120$, $P < 0.0001$) than males (Table 3). Although females weighed more, on average, than males, this difference was not statistically significant ($t = 1.46$, $n = 16$, $P < 0.16$). Other manakins in the genus *Chiroxiphia* showed similar patterns of sexual dimorphism. There was no significant difference in morphology between males in predefinitive plumage and males in definitive plumage (all $P > 0.10$), thus we pooled all males in the following analyses (Table 3). Males had significantly longer tarsi than females in blue manakins, *Chiroxiphia caudata* ($t = –9.2$, $n = 223$, $P < 0.0001$), blue–backed manakins, *Chiroxiphia pareola* ($t = –4.4$, $n = 213$, $P < 0.0001$), lance–tailed manakins, *Chiroxiphia lanceolata* ($t = –6.9$, $n = 156$, $P < 0.0001$), and Yungas manakins, *Chiroxiphia boliviana* ($t = –4.5$, $n = 102$, $P = 0.001$). By contrast, females had significantly longer bills than males in blue manakins ($t = 3.7$, $n = 101$, $P = 0.0003$), blue–backed manakins ($t = 6.6$, $n = 220$, $P < 0.0001$), lance–tailed manakins ($t = 2.2$, $n = 164$, $P = 0.03$), and Yungas manakins ($t = 3.8$, $n = 101$, $P = 0.0002$). Females also weighed significantly more than males in blue–backed manakins ($t = 2.4$, $n = 56$, $P = 0.02$), lance–tailed manakins ($t = 3.2$, $n = 9$, $P = 0.01$), but not in Yungas manakins ($t = 0.85$, $n = 75$, $P < 0.40$). We could not assess sex differences in mass among blue manakins, as too few of the specimens that we examined had mass information available on specimen tags. Instead, we report data from Foster (1987b).

To compare the degree of dimorphism in different morphological traits across *Chiroxiphia* manakins, we calculated a dimorphism index (Greenwood 2003) for each trait as $\log_{10}$(mean male value/mean female value). This dimorphism index results in positive values when males are the larger sex and negative values when females are the larger sex (Fig. 8). The degree of male–biased dimorphism in tarsus length was highly conserved among *Chiroxiphia* manakins (Fig. 8). By contrast, there was considerable interspecific variation in the degree of female–biased dimorphism in bill length and body mass (Fig. 8).

DISCUSSION

Our investigation of morphological variation in a marked population of long–tailed manakins revealed complex patterns of sexual dimorphism in body size and body shape. In particular, adult males had significantly longer tarsi, wings, and elongated tail plumes than females. By contrast, females had significantly longer tails and bills, and weighed significantly more than males. In most cases, young males exhibited trait values intermediate to those of females and adult males. These patterns of dimorphism are unusual in that the identity of the larger sex depends on the trait being considered. In most species, the larger sex is usually larger for all traits considered, even among taxonomic groups where both normal and reverse sexual size dimorphism are common (e.g., Andersson and Norberg 1981; Szekely et al. 2000). Such intraspecific variation in the degree and direction of dimorphism is unexpected, as morphological traits are usually strongly genetically correlated (e.g., Badyaev and Hill 2000; Via and Hawthorne 2005).

These unusual patterns of dimorphism suggest that there has been strong selection for morphological differentiation among male and female long–tailed manakins.

The Direct Competition Hypothesis predicts that selection should favor overall larger body size in males (Table 1). Our findings did not support these predictions, as males were smaller than females for most traits measured, and the few traits in which males were larger than females, namely wing length and tail plume length, are unlikely to be used in agonistic intrasexual interactions. Levels of male–male aggression are relatively low at long–tailed manakin leks, and orderly male queues thought to be reinforced by female preference for low levels of disruption (Foster 1983; Foster 1987a; McDonald 1993).

The Acrobatic Displays Hypothesis predicts that selection should favor long, narrow wings and a small body mass in males (Table 1). Aerodynamic flight efficiency should also favor shorter tail plumes and longer tails (Table 1). Some of our findings are consistent with the Acrobatic Displays Hypothesis. In particular, males had significantly larger wing areas and significantly longer wingspans than females, whereas females had significantly higher wing loading and aspect ratios than males. Thus, relative to females, males have larger, longer, and broader wings that bear less weight per unit area. According to flight aerodynamic theory, this morphology confers males with high flight maneuverability by allowing them to make tight turns and accurate landings (Norberg 1990; Hedenström and Møller 1992). In addition, this phenotype would provide males with high endurance in both hovering and flapping flight (Norberg 1990; Hedenström and Møller 1992). Endurance in hovering flight would benefit males during the performance of dual–male leapfrog displays. Because hovering flight is energetically expensive

(Norberg 1990), the morphology of adult males should allow them to perform these displays at a reduced energetic cost and could thereby enable them to perform higher quality displays, to spend more time displaying, and/or to devote more energy to other important aspects of courtship (McDonald 1989b). Endurance in flapping flight and the ability to make tight turns would benefit males during butterfly displays, which involve both slow labored flights and fast dives to and from the dance perch through flight paths often obstructed by vegetation. Finally, increased ability to make accurate landings would benefit both components of courtship displays. Our findings therefore suggest that sexual selection may have favored the evolution of morphological traits that increase the efficiency of courtship displays in adult male long–tailed manakins. Indeed, one study documented a significant, positive association between the duration of the butterfly display and mating success in long–tailed manakins (McDonald 1989b), suggesting an influence of sexual selection. The number of leapfrog displays showed a similar pattern, although the relationship was not significant (McDonald 1989b). Further analyses of the relationship between flight performance and wing morphology in particular individuals (e.g., Blomqvist et al. 1997) or experimental manipulations of wing shape or wing loading (e.g., Evans and Thomas 1992) would be particularly informative. Sex differences in wing morphology may be paralleled by additional differences in anatomy and physiology. In golden–collared manakins, *Manacus vitellineus*, for example, the wing muscles of males show morphological adaptations to rapid and forceful contractions (Schlinger et al. 2001; Schultz et al. 2001) and wing contractions appear to be modulated in part by sexually dimorphic patterns of sensitivity to sex steroids in spinal cord neural

circuits (Schultz and Schlinger 1999). Such physiological and anatomical adaptations to display behavior remain to be investigated in other manakins.

As documented in other populations (Foster 1977; McDonald 1989a; McDonald 1989b; Arevalo and Heeb 2005), the length of elongated tail plumes increased with male age and was highly sexually dimorphic. By contrast, the length of regular tail feathers decreased with age. These patterns are inconsistent with the Acrobatic Displays Hypothesis, as longer tail plumes increase drag and shorter tails reduce lift (Balmford et al. 1993; Thomas 1993; Norberg 1995). A more likely explanation is that elongated tail plumes in males evolved by sexual selection through female preference for long tails (Prum 1997). Whether tail plume length remains a current target of sexual selection is unclear, although McDonald (1989b) found no evidence of an association between tail plume length and reproductive success in long–tailed manakins. Our finding that, in males, tail length decreases with age whereas tail plume length increases with age is particularly puzzling. Because variation in the size of these traits is likely to be regulated by hormonal changes (Kimball and Ligon 1999; Badyaev 2002), it is difficult to envision how the same hormonal environment could cause a simultaneous increase and decrease in the size of different components of the same trait. Males have been captured with their central rectrices (tail plumes) in various stages of replacement without any signs of molt among other tail feathers (Doucet et al. 2006), and this temporal segregation of molt may enable a divergent pattern of growth for the tail plumes and regular tail feathers. The adaptive significance, if any, of this divergent pattern remains unclear. Limiting resources may result in an energetic trade–off in length between the different types of tail feathers. Contrary to the predictions of such a hypothesis, however, there was a significant positive

relationship between tail length and tail plume length in second–year males ($r = 0.29$, $n = 54$, $P = 0.03$) and no significant relationship between these two traits among males in other age classes (all $P > 0.1$). Another possibility is that shorter tail feathers serve as a signal amplifier by enhancing the appearance of length in the elongated tail plumes (Hasson 1990; Hasson 1991; Arevalo and Heeb 2005). Finally, it is possible that females prefer shorter tails and longer tail plumes in males precisely because these traits impose aerodynamic costs. Thus, short tails and long tail plumes in adult males may serve as sexually–selected handicaps (Zahavi 1975; Zahavi 1977).

Some of our findings were also consistent with ecological hypotheses. The Intersexual Competition Hypothesis predicts that males and females should diverge in traits related to feeding (Table 1). We discovered that females had longer, deeper, and wider bills than males, as well as bills that were wider than predicted by differences in length alone. These differences are even more pronounced when variation in structural body size is taken into account, as females have shorter tarsi than males. Indeed, these differences in bill morphology meet Selander's (1966; 1972) conservative criteria that the only reliable evidence for intersexual niche competition is a sex difference in a trait related to foraging that is both in a direction opposite to that predicted by sexual selection and of a larger magnitude than that predicted by differences in body size alone. The reasoning behind Selander's (1966; 1972) criteria is that sex differences in foraging ecology may be a simple consequence of sexual dimorphism that has evolved through another mechanism, such as sexual selection for large body size in males (e.g., Webster 1997). Further research is needed to determine whether sex differences in bill morphology are accompanied by sex differences in foraging ecology in long–tailed

manakins. Intriguingly, sex differences in foraging ecology have been documented in a closely–related manakin (Marini 1992).

The Reproductive Role Division hypothesis predicts that sexual dimorphism evolves as a consequence of the divergent reproductive roles of males and females (Table 1). This hypothesis makes no general prediction about the direction of dimorphism, but a number of sub–hypotheses have been proposed based on life history characteristics in particular groups (see Andersson 1994a for specific examples). Because females do not perform acrobatic displays and are solely responsible for offspring care, we predicted that reproductive role division would favor large body mass, long, narrow wings, and large, wide bills in females. Our findings supported all of these predictions. First, females weighed significantly more than adult males. Larger female body size may be beneficial in its own right, as it may allow females to defend nests more successfully (Andersson and Norberg 1981), although this interpretation is doubtful for such a small species. Larger female body size may also confer greater fecundity by either of two routes: larger size can allow for the production of larger eggs because it provides more internal space for eggs or because the energy storage capacity required for producing eggs increases more rapidly with body size than do metabolic costs (Downhower 1976; Andersson 1994a). Indeed, female manakins in general (Snow 2004), and long–tailed manakins in particular (Foster 1976b), lay relatively large eggs for such small birds (approximately 15% of their body mass). This egg size is 17% larger than that predicted for a passerine bird and 15% larger than that predicted for a Tyrannid (Rahn et al. 1975).

As further support of the Reproductive Role Division Hypothesis, compared with males, females have shorter, narrower wings that bear more weight per unit area. This

morphology would result in higher load lifting capacity and higher acceleration capacity in flapping flight and terminal speed in gliding dives (Norberg 1990; Hedenström and Møller 1992). In addition to compensating for their higher body mass, high load–lifting capacity could be beneficial to female long–tailed manakins in two ways. First, females must carry the eggs. A 15 % increase in body mass, even if it must be borne only for a short time, will place high energetic demands on flight, and the increased load–carrying capacity of females should partially compensate for this. Similarly, the increased load–carrying capacity of females should facilitate their task of carrying food to the nest. Acceleration capacity in flapping flight and terminal flight speed in gliding dives are typically thought to benefit aerial predators (Andersson and Norberg 1981; Hedenström and Møller 1992). These same flight parameters may increase female efficiency at capturing insects in flight. Although most manakins are largely frugivorous, females supplement their own diets with insects to a larger extent than do males, and they feed their offspring an even larger proportion of insects than they ingest themselves (Snow 2004).

That females had longer, wider bills than males may also support the Reproductive Role Division hypothesis. Manakins, like many frugivorous birds, are gape–size limited (Wheelwright 1985; Levey 1987; Snow 2004). Accordingly, there is a close correspondence between gape size and both the mean and maximum diameter of fruits eaten (Wheelwright 1985; Snow 2004). When feeding offspring, females either carry fruit in their bills or regurgitate previously ingested items (Foster 1976b; Snow 2004). A larger gape would allow females to ingest larger fruits or to carry more fruit in their bills than males. Because take–off and landing are costly for birds with high wing

loading, females should select the largest fruits available to maximize energy gain (Norberg 1990), and this would apply both to ingesting food for themselves and to feeding offspring. Moreover, having larger bills provides females with a greater potential resource base, as it allows them to eat larger fruits without compromising their ability to eat small fruits (Wheelwright 1985; Levey 1987; Avery et al. 1993; Rey et al. 1997), and should also allow them to capture flying insects more effectively (e.g., Keast et al. 1995). Hence females, and, indirectly, their offspring, may be better equipped to deal with periods of food scarcity caused by stochastic events (Foster 1976a). Finally, compelling recent studies suggest that predation intensity has had a important influence on life history evolution in birds (Martin et al. 2000; Martin 2002). In particular, these studies suggest that higher predation pressure in the tropics constrain the rate at which parents can deliver food to their young (Martin et al. 2000; Martin 2002). Larger bills should reduce the number of foraging trips required to deliver food to offspring, thereby reducing the exposure of females and nestlings to potential predators. Because sexual dimorphism in bill length remains highly significant even when controlling for body mass ($t = 6.33$, $n = 175, 170$, $P < 0.0001$), our data suggest that large bill size in females is not simply a consequence of the energetic demands of higher body mass in females.

Taken together, our findings suggest that both ecological and sexual selection pressures may be responsible for the evolution of sexual dimorphism in long–tailed manakins. In particular, our findings were consistent with some predictions of the Acrobatic Displays, Intersexual Competition, and Reproductive Role Division hypotheses. Our data do not allow us to directly attribute patterns of dimorphism to a particular mechanism, as further investigation of natural and sexual selection pressures on

morphological traits in each sex will be required to confirm our interpretations (e.g., Price 1984). Nonetheless, our data identify likely hypotheses that clearly warrant further investigation. Moreover, our findings highlight the importance of considering both ecological and sexual selection hypotheses in investigations of sexual dimorphism. Many studies focus on only one or the other of these mechanisms (Hedrick and Temeles 1989), and in a lek–mating group like manakins, sexual selection might be favored as the key driving force behind the evolution of dimorphism. Our findings suggest, however, that ecological mechanisms, including Intersexual Competition and Reproductive Role Division, may be equally important. Indeed, sexual selection and ecological selection may reinforce each other. For example, whereas the Acrobatic Displays Hypothesis predicts small body size and long, narrow wings in males, the Reproductive Role Division hypothesis predicts a nearly opposite pattern in females. Similarly divergent influences of sexual and ecological selection might be expected in other lek–mating species, as the reproductive roles of males and females differ strongly by definition in this type of mating system (Bradbury 1981; Höglund and Alatalo 1995). Such opposing selection pressures are consistent with the idea that patterns of dimorphism reflect the sum of natural and sexual selection pressures acting separately on males and females (Price 1984; Schluter et al. 1991; Merilä et al. 1997; Badyaev et al. 2000; Badyaev 2002), and may in fact be a necessary precursor to the evolution of dimorphism.

Males in predefinitive plumage generally exhibited trait values intermediate to those of females and males in definitive plumage. Indeed, morphological variation alone was sufficient to categorize most (84%) individuals into appropriate sex and/or age categories. For all feather traits and body mass, younger males were more similar to

females than to older males. Thus, dimorphism in these traits increases with male age. Because morphological changes in young males were paralleled by striking changes in plumage coloration and behavior converging on that exhibited by adult males (Doucet et al. 2006), we propose that ontogenetic changes in male body mass and wing morphology reflect the increasing influence of sexual selection for courtship display efficiency. Sexual dimorphism in wing shape and body mass may reflect a compromise between different flight characteristics in manakins. Flight endurance and maneuverability will provide the greatest benefit to those few males that invest the most in, and stand to gain the greatest benefits from, courtship displays. Yet, this morphology may be costly among males who do not hold dominant status at leks and cannot therefore display for females and compete for copulations. Similarly, load–lifting capacity and flight speed may benefit some components of female fitness at the expense of others. Their higher wing loading, for example, likely makes them more vulnerable to predation due to slower take–off speeds (Kullberg et al. 2002a; Kullberg et al. 2002b; Kullberg et al. 2005). Such trade–offs may in part explain why the phenotype of young males is intermediate to that of females and adult males.

Alternative explanations have been proposed to explain patterns of dimorphism similar to those we documented. For example, some studies suggest that smaller bill size can enhance vocal performance in male birds by allowing them to sing trilled songs at a faster rate or higher bandwidth (Podos et al. 2004a; Podos and Nowicki 2004). This mechanisms is unlikely to be important in manakins, however, as males dot no typically sing trilled vocalizations (Trainer and McDonald 1993; Snow 2004). Further research is needed to determine whether other types of vocalizations are subject to similar

performance constraints (Podos et al. 2004b). If so, sexual selection for vocal performance and, indirectly, small bill size in males, may serve as an additional reinforcing mechanism explaining the patterns of dimorphism documented in this study. A second alternative explanation is that the lower body mass of adult males is not an adaptive response to selection for display efficiency, but rather reflects the costs of sexual advertisement and display (McDonald 1989a). McDonald (1989a) documented similar patterns of dimorphism and age–graded changes in body mass in another population of long–tailed manakins. In that population, however, older males gained weight during the non–breeding season, such that they did not weigh significantly less than females. Such a breeding–season induced weight reduction in males could reflect the energetic costs of extended periods of advertisement and display (McDonald 1989a; McDonald 1989b; Andersson 1994b). Although this alternative hypothesis deserves further investigation, it does not necessarily preclude an adaptive advantage of low body mass as predicted by the Acrobatic Displays Hypothesis.

Many of the patterns of sexual dimorphism that we documented in long–tailed manakins were paralleled in other *Chiroxiphia* manakins. In particular, males had significantly longer tarsi whereas females had significantly longer bills in all four species. Females also weighed significantly more in two of the three species for which these data were available, but not in Yungas manakins or blue manakins (Foster 1987b). We did not measure the wing lengths of museum specimens. However, Payne (1984) reports male–biased dimorphism in wing length four species of *Chiroxiphia* manakins. In our wild–caught long–tailed manakins, wing length was positively correlated with wing area ($r = 0.36$, $n = 49$, $P = 0.009$), and this likely applies to other *Chiroxiphia* manakins. Given

that females generally weigh more than males, their wing loading will probably be greater than that of males. Such similarities in patterns of dimorphism are interesting in light of the fact that *Chiroxiphia* manakins also share many similarities in life history traits. Four of the species are known to exhibit a lek–based mating system with cooperative male displays, female–only parental care, and delayed male plumage maturation (Foster 1977; Foster 1987a; Snow 2004). Further investigation of possible associations between the degree of wing–length, bill–length, and body mass dimorphism and interspecific variation in courtship displays and feeding ecology would be particularly informative, both within *Chiroxiphia* and among the Pipridae. In *C. caudata*, for example, intrasexual male aggression at display perches is more common (Foster 1981; Foster 1983) and selection for competitive ability may drive male body size away from that optimal for display efficiency. These opposing selection pressures may explain the absence of sexual dimorphism in body mass for *C. caudata* (Foster 1987a).

Payne (1984) first suggested that reversed sexual size dimorphism may have evolved to facilitate aerial displays in manakins. Our findings within long–tailed manakins and among *Chiroxiphia* manakins offer partial support for this hypothesis. Payne (1984) also suggested that reversed size dimorphism was more common among smaller manakin species. However, *Chiroxiphia* manakins, all of which show clear patterns of reversed dimorphism in some traits, are among the largest in the family (Snow 2004). No study has considered the adaptive significance of sexual dimorphism in bill size and shape among the Pipridae. If, as suggested by our findings in *Chiroxiphia* manakins, gape–size limited frugivory and female–only parental care favor larger bill dimensions in females, other species that share these life history traits should exhibit

similar patterns of dimorphism in bill size. Our hypothesis receives anecdotal support in at least one group of birds. Among the bowerbirds (Ptilonorhynchidae), a predominantly frugiviorous family, females have relatively larger bills in polygynous species with female–only parental care than in monogamous species with bi–parental care (Frith and Frith 2004). As partly frugivorous birds exhibiting variation in mating systems, the bowerbirds, birds of paradise, tyrannid flycatchers, manakins, and cotingas offer several opportunities for comparative tests of this hypothesis.

We have documented unusually complex, age–specific patterns of male–biased and female–biased sexual size dimorphism in long–tailed manakins and other members of the genus *Chiroxiphia*. Overall body size and wing shape are likely to be constrained by features common to all of these species. Manakins are primarily forest–dwelling birds (Snow 2004), and flying in environments cluttered with vegetation favors the evolution of relatively short wings, regardless of taxonomic affiliations (Norberg 1990). Similarly, manakins forage primarily by sallying for fruits (Snow 2004). This foraging behavior, along with a disproportionately wide gape, allows manakins to exploit fruits to a greater extent than larger but less specialized frugivores such as tanagers (Snow 2004). However, aerial sallies are energetically expensive and make particular demands on body size and wing morphology (Norberg 1990). Our findings suggest that, despite these general constraints on body morphology, differing and often opposing selective forces driven by sexual divergence in life history traits has led to the evolution of complex, age–related patterns of sexual dimorphism in long–tailed manakins. Moreover, our data are consistent with predictions of both Sexual Selection Hypotheses and Ecological Hypotheses. Our findings suggest that both sexual selection and ecological selection have played an

important role in shaping the sexually divergent morphologies of manakins. The evolution of dimorphism in manakins has likely been strongly influenced by their lek–based mating system. Such a mating system places intense sexual selection pressure on males while simultaneously segregating the reproductive roles of males and females. The subsequent evolution of striking sex differences in life history traits may then favor morphological divergence through differences in the relative influence of natural and sexual selection on male and female morphologies.

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Table 1. Predicted direction of dimorphism for morphological traits in long–tailed manakins based four non–mutually exclusive hypotheses. See text for explanations and sources of hypotheses.

	Sexual Selection Hypotheses		Ecological Hypotheses	
	Direct Competition	**Acrobatic Displays**	**Intersexual Competition**	**Reproductive Role Division**
Overall influence	Favors overall large body size in males	Favors small body size and long, broad wings in males	Favors divergence between the sexes for feeding–related traits	Consequence of different reproductive roles of males and females
Predictions				
Wing size and shape	Large male wings	Long, broad male wings	Equivocal	Long, narrow female wings
Body mass	Large male body mass	Small male body mass	Equivocal	Large female body mass
Bill size and shape	Large male bills	Equivocal	Males ≠ females	Large, broad female bills
Tail plume length	Long male plumes	Short male plumes	Equivocal	Equivocal
Tail length	Long male tails	Long male tails	Equivocal	Equivocal
Tarsus length	Long male tarsi	Equivocal	Equivocal	Equivocal

Table 2. Flight parameters, their associated measures of performance, and the sizes or magnitudes of four morphological traits that should enhance performance for each specific flight parameter (see Hedenström and Møller 1992). The final column indicates which sex is predicted to show higher performance for each parameter based on the morphological variation documented in this study.

Flight parameter	Performance	Mass	Wingspan	Wing area	Aspect ratio	Wing loading	Sex
1. Min turning radius	Maneuverability	Low	No effect	Small	Low	Low	Males
2. Roll acceleration	Maneuverability	High	Long	Large	Unknown	High	Equivocal
3. Min gliding speed	Landing accuracy	Low	No effect	Large	Low	Low	Males
4. Min sink speed in gliding flight	Endurance	Low	Long	Large	Unknown	High	Males
5. Min power in hovering flight	Endurance	Low	Short	No effect	Low	Low	Males
6. Power in flapping flight	Endurance	Low	Short	No effect	Low	Low	Males
7. Best glide ratio	Efficient flight	No effect	Long	Large	High	High	Equivocal
8. Terminal speed in gliding dive	Speed	High	Long	No effect	High	High	Females
9. Max linear accel. in flapping flight	Speed	High	Long	No effect	High	High	Females
10. Climb rate in flapping flight	Load–lifting capacity	High	Long	No effect	High	High	Females

Table 3. Means ± standard deviations of morphological traits in museum specimens of male and female *Chiroxiphia* manakins.

Species	Specimens Measured‡		Tarsus length (mm)		Bill length (mm)		Mass (g)	
	Male	Female	Males	Females	Males	Females	Males	Females
blue manakin (*C. caudata*)	142	88	21.12 ± 0.89	20.04 ± 0.78*	6.66 ± 0.32	6.83 ± 0.35*	22.60 ± 1.15	22.65 ± 1.61
blue–backed manakin (*C. pareola*)	172	51	19.12 ± 0.94	18.42 ± 1.05*	6.28 ± 0.41	6.72 ± 0.43*	16.91 ± 1.81	18.17 ± 2.01*
long–tailed manakin (*C. linearis*)	96	26	18.78 ± 0.57	17.87 ± 0.58*	5.81 ± 0.35	6.15 ± 0.31*	18.33 ± 1.97	19.72 ± 0.98*
lance–tailed manakin (*C. lanceolata*)	136	30	18.95 ± 0.61	18.04 ± 0.78*	6.44 ± 0.34	6.59 ± 0.38*	17.17 ± 0.32	20.60 ± 2.09*
Yungas manakin (*C. boliviana*)	93	12	18.86 ± 0.65	18.05 ± 0.73*	5.83 ± 0.33	6.22 ± 0.30*	17.81 ± 1.38	18.21 ± 1.34

‡Sample sizes differ for different morphological measurements (see text for details).

* Identifies statistically significant ($P < 0.05$) differences between males and females (see text for details).

FIGURE CAPTIONS

Figure 1. Schematic diagram illustrating aspects of wing morphology measurements in the long–tailed manakin. The fully outstretched wing was traced with the wing positioned ventral side down. The tracing was shorter than the wing length because of the body area between the proximal edge of the wing and the sternum, known as the root box (dashed line). This rectangular area was quantified by multiplying the width of the wing where it meets the body by the difference between total wing length and the length of the wing tracing. The root box area was then added to the traced wing area, resulting in one half of the wing area (shaded in grey). The total wing area was thus twice this value.

Figure 2. Box plots showing differences in morphological traits among male long–tailed manakins in different age classes. Horizontal lines in box plots show 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles. With the exception of tarsus and bill length, all traits vary significantly with male age (see text for statistical details).

Figure 3. Box plots showing sex differences in morphological traits in long–tailed manakins. Horizontal lines in box plots show 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles. All traits are significantly different between sexes (see text for statistical details).

Figure 4. Scatterplot of canonical axes 1 and 2 of a Discriminant Function Analysis in long–tailed manakins where sex and age categories were the classification variables and six morphological variables (wing length, tarsus length, bill length, tail length, tail plume length, and mass) were the measurement variables. Shown are females (open circles),

second–year males (light grey circles), third–year males (medium grey circles), fourth–year males (dark grey circles), and fifth–year or older males (black circles).

Figure 5. Box plots showing differences wing in morphology among male long–tailed manakins in different age classes. Horizontal lines in box plots show 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles. All traits vary significantly with male age (see text for statistical details).

Figure 6. Box plots showing sex differences in wing morphology in long–tailed manakins. Horizontal lines in box plots show 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles. All traits are significantly different between sexes (see text for statistical details).

Figure 7. Three–dimensional morphospace of wing size and shape, bill size and shape, and body mass showing how males (filled circles) and females (open circles) occupy different areas of the morphospace (see text for details of PC axes).

Figure 8. Sexual dimorphism indexes for three morphological traits based on measurements from museum specimens of *Chiroxiphia* manakins. Positive values are obtained for traits where males are larger than females and negative values are obtained for traits where females are larger than males (see text for details of calculations). Mass data for *C. caudata* from (Foster 1987b).

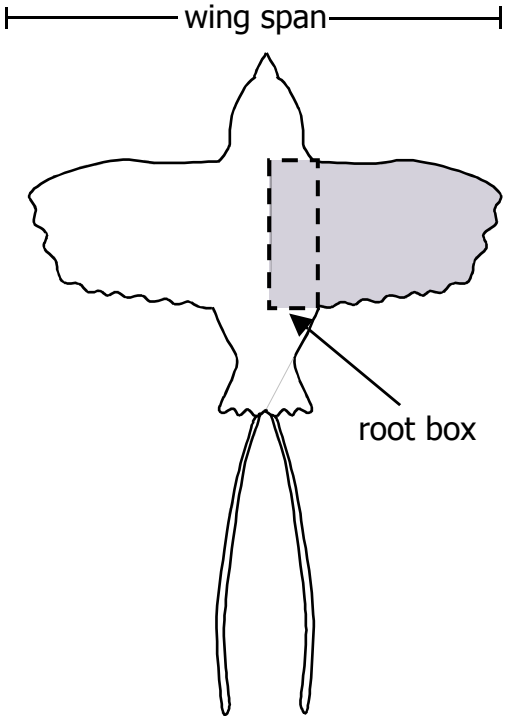

Figure 1.

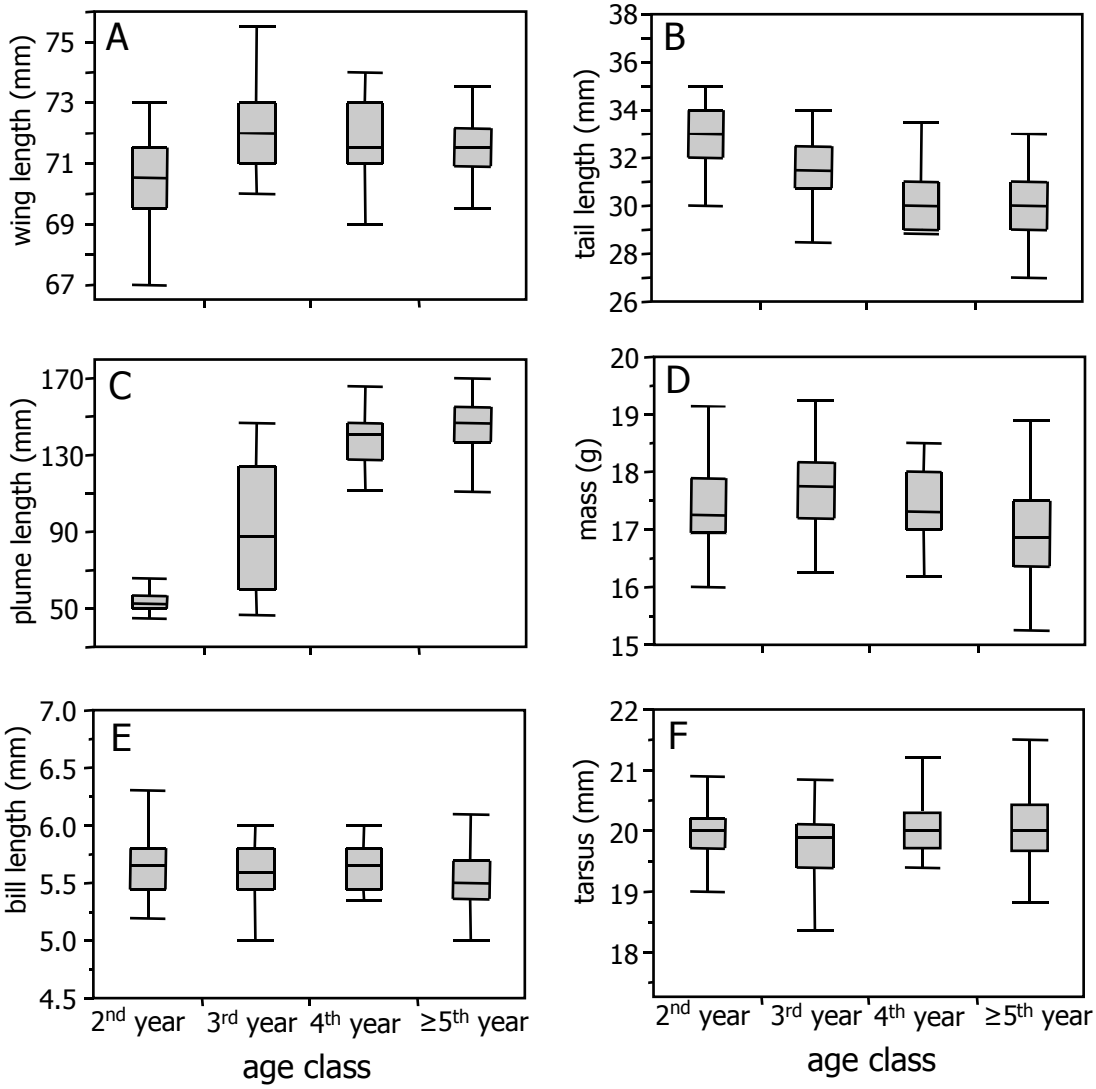
A
wing length (mm)
75
73
71
69
67
B
tail length (mm)
38
36
34
32
30
28
26
C
plume length (mm)
170
130
90
50
D
mass (g)
20
19
18
17
16
15
E
bill length (mm)
7.0
6.5
6.0
5.5
5.0
4.5
F
tarsus (mm)
22
21
20
19
18
2nd year
3rd year
4th year
≥5th year
age class

Figure 2.

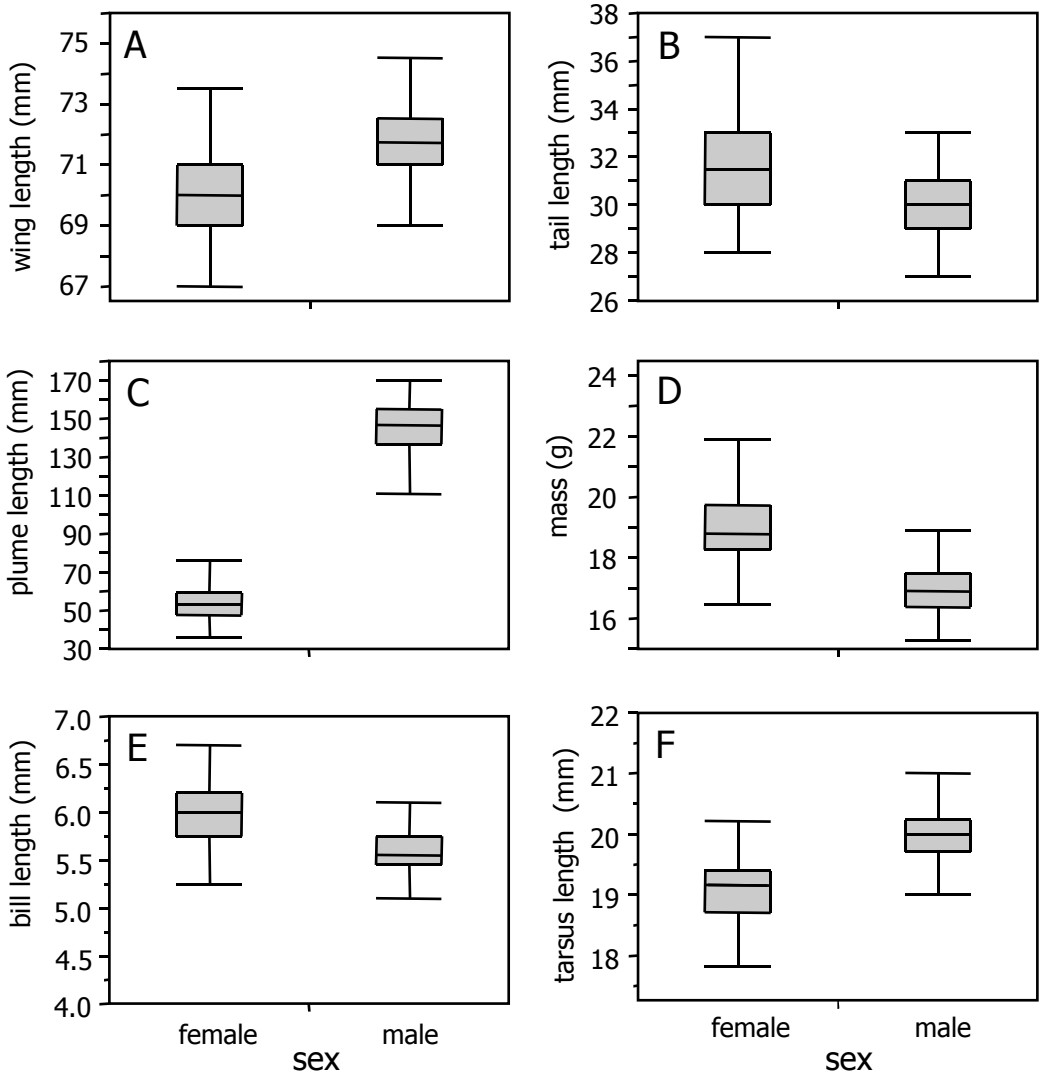
A
wing length (mm)
75
73
71
69
67
B
tail length (mm)
38
36
34
32
30
28
26
C
plume length (mm)
170
150
130
110
90
70
50
30
D
mass (g)
24
22
20
18
16
E
bill length (mm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
F
tarsus length (mm)
22
21
20
19
18
female
male
sex
female
male
sex

Figure 3

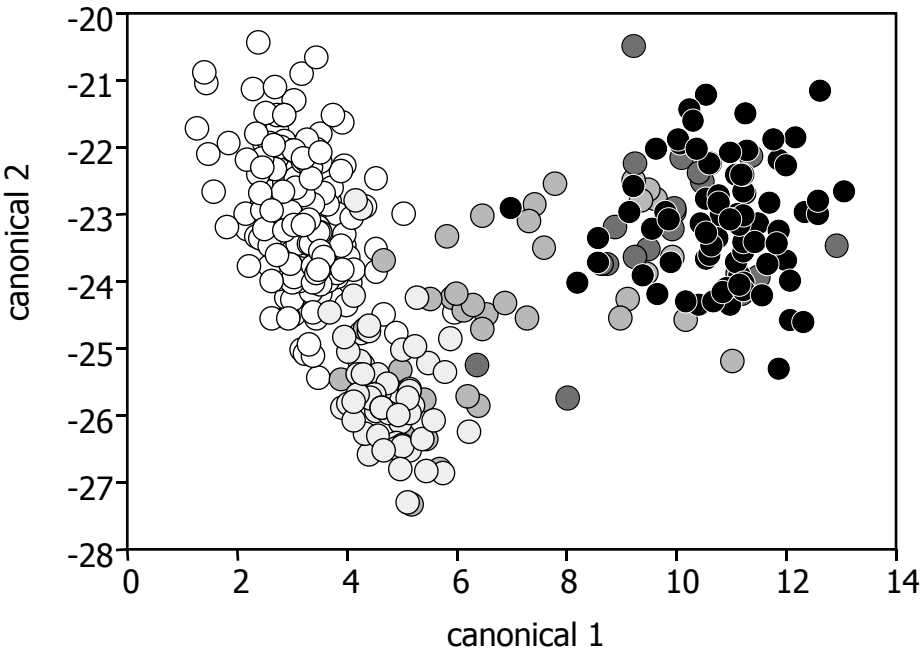

Figure 4

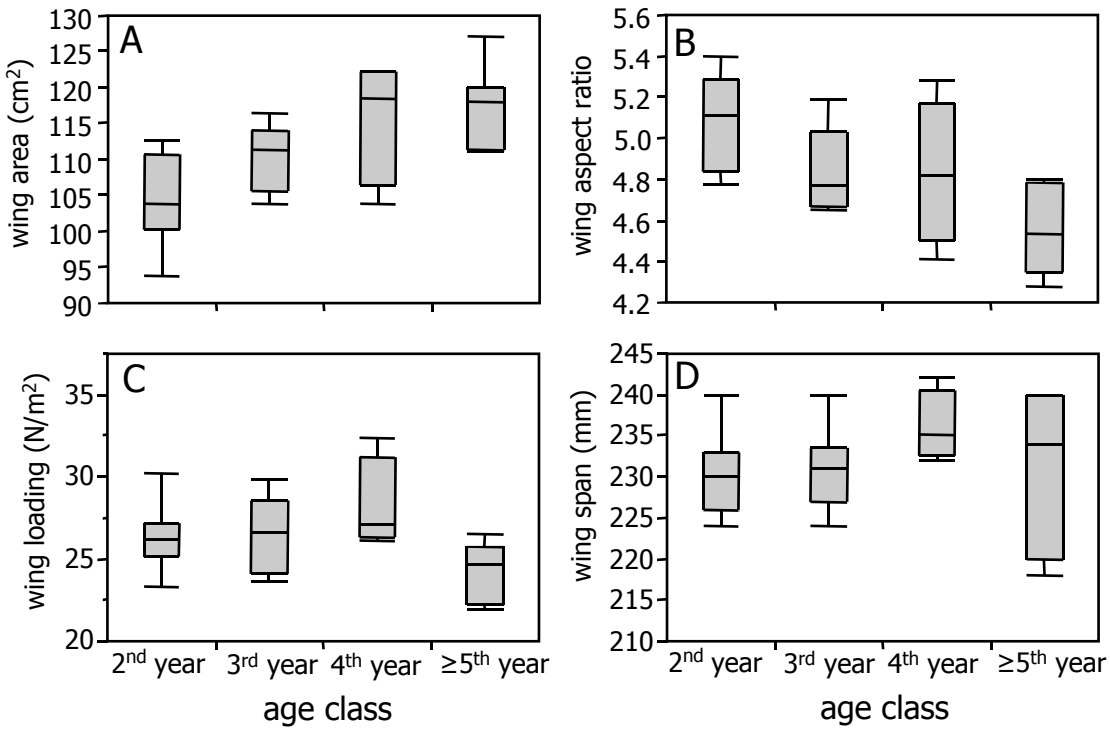
A
wing area (cm²)
130
125
120
115
110
105
100
95
90
B
wing aspect ratio
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
C
wing loading (N/m²)
35
30
25
20
D
wing span (mm)
245
240
235
230
225
220
215
210
2nd year
3rd year
4th year
≥5th year
age class

Figure 5

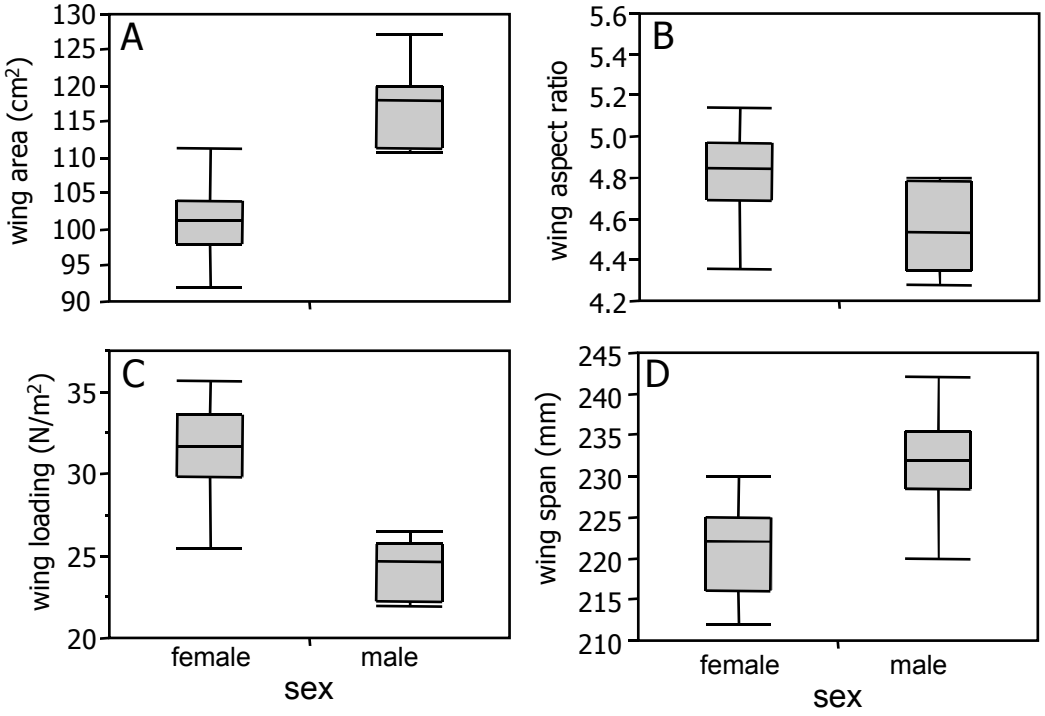
A
wing area (cm²)
130
125
120
115
110
105
100
95
90
B
wing aspect ratio
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
C
wing loading (N/m²)
35
30
25
20
female
male
sex
D
wing span (mm)
245
240
235
230
225
220
215
210
female
male
sex

Figure 6

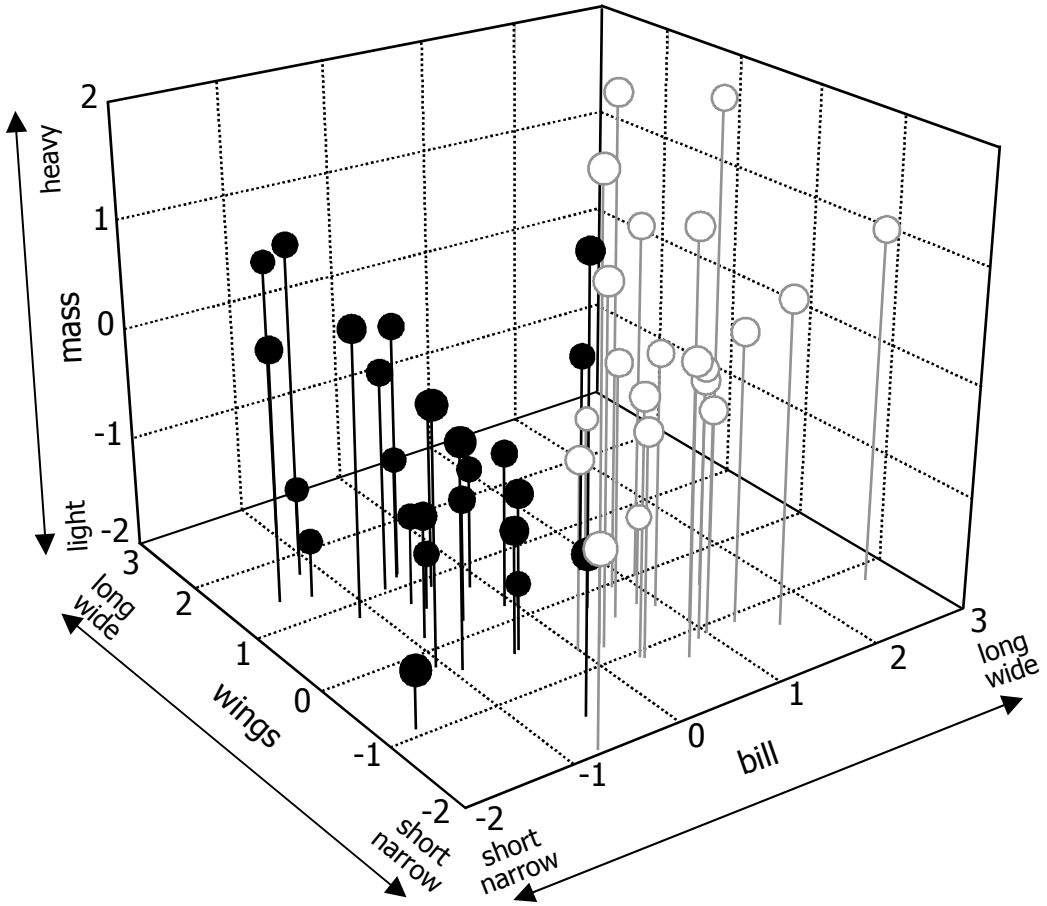
mass
heavy
light
2
1
0
-1
-2
wings
long
wide
short
narrow
3
2
1
0
-1
-2
bill
long
wide
short
narrow
3
2
1
0
-1
-2

Figure 7

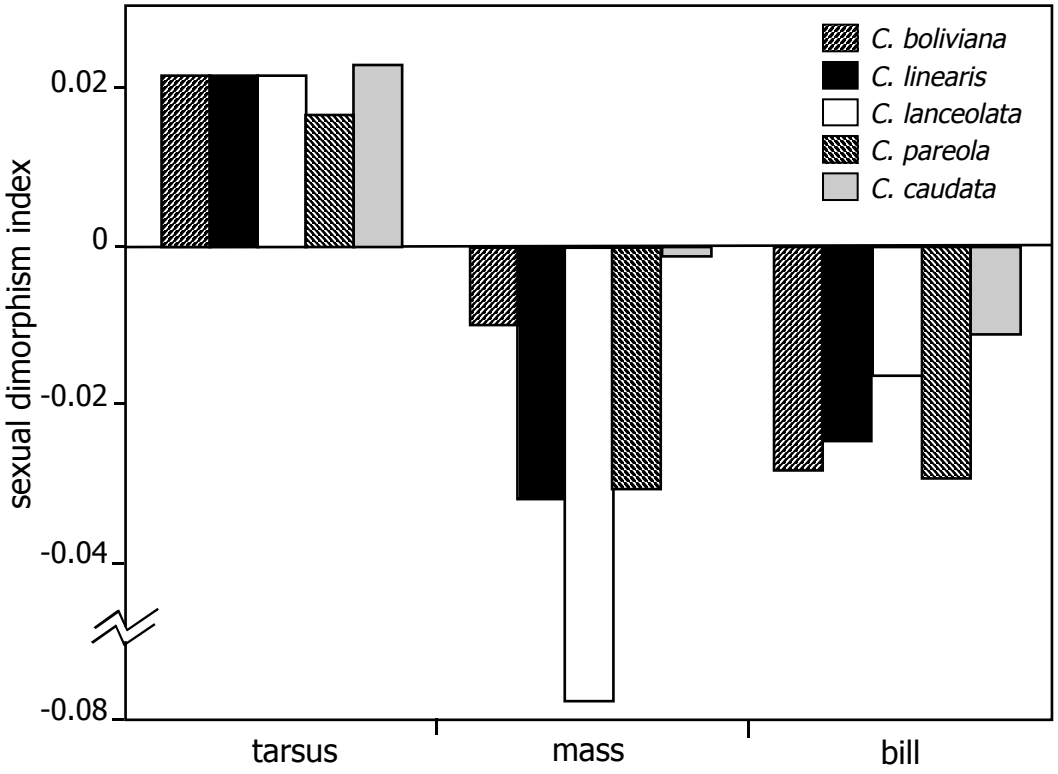
C. boliviana
C. linearis
C. lanceolata
C. pareola
C. caudata
sexual dimorphism index
0.02
0
-0.02
-0.04
-0.08
tarsus
mass
bill

Figure 8

CHAPTER 3. LEKS, LIGHT, AND LOOKS: DOES THE VISUAL ENVIRONMENT INFLUENCE DISPLAY CONSPICUOUSNESS IN LONG–TAILED MANAKINS?

ABSTRACT

The conspicuousness of a visual signal depends in part upon the environment in which it is perceived. In this study, we examined the influence of light environment and visual background on the conspicuousness of male and female plumage coloration in long–tailed manakins. We also investigated whether males adjusted the location or timing of their displays to take advantage of particular light environments. We used reflectance spectrometry to quantify the plumage coloration of males (n = 62) and females (n = 59) as well as the coloration of the vegetation comprising the visual background at display sites. We used irradiance spectrometry to measure light environments at display perches and at nearby control sites in the forest understory. We analyzed coloration data using both principal components analysis and an avian visual color space model. Both methods revealed striking differences in the conspicuousness of males and females. PCA revealed that the olive–green coloration of females represents a subset of the colors comprising the visual background of the forest understory, whereas the red, blue, and black coloration of males occupies a larger portion of the PCA color space and contains unique elements that are not found in the visual background. The color space model further revealed that females exhibit low levels of contrast against the background and low levels or contrast between plumage regions. Males, on the other hand, exhibit high levels of contrast against the background and contrast between plumage regions. Variation in both the visual background and the light environment had a pronounced influence on the conspicuousness of male and female plumage patterns. Male manakins did not, however, adjust the location or timing of their displays to take advantage of particular light environments. Instead, they performed the majority of their displays in forest shade, the

most common light environment. Our findings suggest that by displaying in forest shade, long–tailed manakins may optimize the short–distance conspicuousness of male plumage ornaments while minimizing the long–distance conspicuousness of male ornaments and the short– and long–distance conspicuousness of female plumage patterns. By so doing, male manakins can present colorful displays to nearby females while minimizing their risk of predation from distant predators. Our findings contribute to a growing body of evidence suggesting that the perceptual environment has an important influence on the evolution of visual signals used in social communication.

INTRODUCTION

In many animals, the coloration or patterning of the skin, fur, feathers, or scales serve as visual communication signals. The perception of a visual signal by potential receivers will be influenced by the structural or biochemical properties of the signal, the light environment in which it is transmitted, the background against which it is viewed, the visual capabilities of the signal receiver, and further psychophysical processing of the signal that occurs once it has been detected (Lythgoe 1979; Endler 1990; Guilford and Dawkins 1991; Endler and Basolo 1998; 1998; Vorobyev et al. 2001; Théry 2006). Whether or not a signal is conspicuous or cryptic depends on the surrounding light environment and the background against which the signal is viewed (Endler 1990). Consequently, even the most colorful signals, though undoubtedly conspicuous in some situations, can appear remarkably cryptic at other times. This concept will be familiar to anyone who has watched a flock of colorful parrots all but disappear as they alight in a rainforest canopy.

Important developments in signal design theory (e.g., Endler 1992; Endler 1993b), visual modeling (e.g., Vorobyev and Osorio 1998; Vorobyev et al. 1998), and spectrometric measurement and analysis (Endler 1990; Andersson and Prager 2006; Montgomerie 2006) have motivated new studies in the role of the perceptual environment in shaping the evolution of colorful signals. In polymorphic bluefin killifish (*Lucania goodei*), for example, variation in light environment predicts the relative abundance of different color morphs, suggesting that selection for efficient communication drives the evolution of different color morphs in different light environments (Fuller 2002). In a comparative analysis of Australian birds, differences in color between closely–related

pairs of species were associated with differences in habitat (open versus closed) but not differences in distribution (sympatric versus allopatric), suggesting that variation in the light environment has a greater influence on plumage color evolution than selection for species recognition (McNaught and Owens 2002). Among agamid lizards, males were more conspicuously colored than females in closed habitats, suggesting that coloration may have been molded by sexual selection, but when coloration and environment were examined separately for each sex, coloration was associated with habitat openness, suggesting that natural selection also played an important role (Stuart–Fox and Ord 2004). Light environment also varies along a vertical gradient in forests (Endler 1993a; Heindl and Winkler 2003b), and a comparative investigation of an avian rainforest community revealed that patterns of plumage coloration differed across vegetation strata from the understory to the canopy (Gomez and Théry 2004). In particular, male plumage was more conspicuous than female plumage at each height, and some components of color appeared to be under natural selection for crypsis, whereas other components appeared to be under sexual selection for conspicuousness (Gomez and Théry 2004). Finally, a recent comparative study revealed that among Neotropical manakins, increasing sexual dichromatism was positively associated with plumage contrast against the background in males but not females, suggesting that sexual selection favors the evolution of conspicuous male coloration that diverges from background coloration (Doucet et al. 2006b).

While the studies outlined above clearly suggest that the perceptual environment has an important influence on the evolution of visual signals, they also highlight two potential sources of conflict in the evolution of signal design. First, selection for

detectability and assessment by conspecifics should favor the evolution of conspicuous signals, whereas predation pressure should favor the evolution of cryptic signals (Endler 1978; Endler 1991). Second, if the visual signal evolved by sexual selection and if such selection acts more strongly on males than on females then males should experience selection for conspicuous coloration, whereas females should experience selection for cryptic plumage (Endler and Théry 1996; Götmark et al. 1997). Potential resolutions to these conflicts include the evolution of ornaments that can be displayed facultatively, such as expandable dewlaps in anoles (e.g., Macedonia et al. 2000), concealable crests in birds (e.g., Graves 1990), and motile pigment cells in fishes (Fujii 2000) and squids (e.g., Hanlon et al. 1994), or the evolution of ornaments that are relatively inconspicuous to predators because of differences in their visual systems (Hastad et al. 2005). These potential solutions are relatively rare, however, and animals more commonly adjust their conspicuousness by varying the timing and/or location of their displays. For example, guppies (*Poecilia reticulata*) adjust the timing and location of their displays such that their coloration patterns are more conspicuous during courtship and less conspicuous at times of high predation risk (Endler 1991).

Species with lek mating systems tend to display at fixed locations and thereby offer a tractable system in which to investigate the influence of the visual environment on the conspicuousness of colorful male ornaments. Endler and Théry (1996) discovered that each of three neotropical, lek–mating species displayed in a subset of the light environment that enhanced the conspicuousness of their coloration patterns at the time of display. Heindl and Winkler (2003a) showed that male wire–tailed manakins (*Pipra filicauda*) displayed in light environments that enhanced their conspicuousness at short

viewing distances while reducing their conspicuousness at long viewing distances, suggesting a possible resolution of the trade–off between performing conspicuous courtship displays while avoiding detection by predators. In a separate study, Heindl and Winkler (2003b) showed that each of four sympatric species of manakins displayed at a height in the rainforest where the corresponding light environment enhanced some component of its plumage conspicuousness. Finally, Uy and Endler (2004) found that by clearing display courts on the ground, male golden–collared manakins (*Manacus vitellinus*) modified their visual background in a manner that increased their plumage contrast and hence conspicuousness.

In this study, we modeled how variation in light environment and visual background influenced plumage conspicuousness in male and female long–tailed manakins (*Chiroxiphia linearis*). We also investigated whether males of this species varied the location or timing of their displays to take advantage of particular visual environments. Long–tailed manakins are strikingly sexually dichromatic: males in definitive plumage have a bright red crown, a sky blue mantle, and contrasting black body and flight feathers, whereas females are olive green throughout. Before attaining this definitive plumage in their fifth year, young males progress through several age–specific intermediate plumages (Doucet et al. 2006a). Long–tailed manakins follow a lek–based mating system where several males gather at dispersed lek arenas to display to females. Each lek is characterized by a generally age–graded dominance hierarchy (Foster 1977; McDonald 1989a; McDonald 1989b). Only the two most dominant males at each lek perform courtship displays for females (Foster 1977; McDonald 1989a; McDonald 1989b). These males advertise to females by singing “toledo” duets from

within the subcanopy (Trainer and McDonald 1993). If these duets succeed in attracting a female to the lek, all three birds fly to a display perch, typically a horizontal vine near the ground, and the males begin to perform their cooperative displays. During leapfrog displays, the two males cartwheel over one another in a highly coordinated fashion. During butterfly flights, the two males alternately or simultaneously perform slow, labored flights and fast dives between the dance perch and vines or branches up to 20 m away (Foster 1977; McDonald 1989a; McDonald 1989b). At each lek arena, the vast majority of these displays are performed on a single perch (McDonald 1989a), which we term the primary display perch. However, some leks also encompass secondary and tertiary display perches.

We used irradiance and reflectance spectrometry, visual modeling, and behavioural observations of long–tailed manakins to achieve five primary objectives. First, we quantified and compared the reflectance of male and female plumage and the reflectance of the vegetation comprising the visual background of manakin display areas. Second, we used irradiance spectrometry to identify and quantify light environments at display perches and at control sites. Third, we used an avian tetrachromatic color space model to document how variation in the light environment and the visual background influences the conspicuous of plumage coloration in male and female manakins. Fourth, we investigated whether manakins adjust the location of their displays to enhance their plumage conspicuousness. Finally, we investigated whether manakins adjust the timing of their displays to enhance their plumage coloration.

METHODS

Study site

We studied long–tailed manakins from March to July 2003–2004 in Santa Rosa National Park, Guanacaste Conservation Area, Costa Rica (10˚ 40’ N, 85˚ 30 W). This region of Costa Rica exhibits a pronounced seasonality, with a dry season extending from December to May, and a rainy season extending from June to November. Our study site was located in an isolated tract of bottomland moist tropical forest. This forest, locally known as the Bosque Humedo, consists of primarily evergreen trees and retains a thick canopy cover year–round (Janzen 1983). The breeding season of long–tailed manakins extend from February to August, with a marked peak in male display activity and female lek visitation between March and June (Foster 1977; McDonald 1989a; McDonald 1993). At our study site, male long–tailed manakins begin to advertise at sunrise and display throughout the morning. Display activity is sparse and sporadic in the afternoons.

Reflectance of manakin plumage patches

We captured long–tailed manakins by placing mist nets near active display perches. We marked each individual with a unique combination of one numbered aluminum leg band and up to three colored leg bands. At the time of capture, we recorded standard morphometric measurements and collected plumage reflectance data from each individual. We used a USB2000 reflectance spectrometer and PX–2 pulsed xenon lamp (Ocean Optics, Dunedin, FL) to collect reflectance readings. Light was delivered from the light source to the measurement surface, and from the measurement surface to the spectrometer, via a bifurcated fiber–optic cable and reflectance probe. The probe was

encased in a black rubber sheath that excluded external light from the measurement area and maintained the probe at a fixed distance to, and perpendicular from, the measurement surface. We measured five body regions for each bird (crown, mantle, tail, wing, breast), taking five readings from each region. Each reading was composed of 20 readings collected in rapid succession and averaged by the operating software. Reflectance was calculated relative to a WS–1 white standard, which reflects > 97% of incident light (Ocean Optics).

Reflectance of the visual background

Using the equipment and procedures described above for plumage patches, we measured the reflectance of objects comprising the visual background of manakin display sites. At each of 14 leks, we measured the reflectance of the bark of the primary display perch (n = 14), the reflectance of two dead leaves arbitrarily selected from the leaf litter below the perch (n = 28), the reflectance of the bark of all trees with dbh > 5 cm that were within a 2 m radius of the display perch (n = 18), and the reflectance of one green leaf from each of the four saplings nearest to the display perch (n = 56). As with plumage patches, we collected five readings from each object.

Available light environments

Manakins perform their coordinated dual–male displays on low, horizontal vines or branches in the forest understory (McDonald 1989b). Based on opportunistic observations and scheduled behavioral observations (see *Light environments and timing of displays* below), we noted that three types of light environment were available for

manakin displays: forest shade, small gaps, and cloudy (sensu Endler 1993a). In the first half of the breeding season, which coincides with the dry season, sunny conditions prevail and only forest shade and small gap light environments were available for display. Forest shade light environments characterize the shady understory of closed–canopy forests in sunny conditions (Endler 1993a). Small gaps interrupt forest shade where small breaks in the canopy allow small patches of direct sunlight to reach the understory (Endler 1993a). Cloudy light environments became available during the rainy season, when intermittent sunny, cloudy, and rainy weather, provided access to all three light environments. Cloudy light environments characterize the forest understory when the sun is completely obstructed by clouds (Endler 1993a).

To incorporate the effect of variation in the light environment into our visual models, we measured these light environments at 24 primary display perches. At each display perch, we measured the color of ambient light spectra with a USB2000 spectrometer and a cosine corrected fiber optic irradiance probe with a 180° angle of acceptance and a measurement surface of 6 mm in diameter (CC–3–UV, Ocean Optics). At each measurement location, we calibrated the spectrometer with a calibration light source of known color temperature (LS–1–CAL, Ocean Optics). We collected five readings per location with the probe oriented skyward (perpendicular to the ground) at the height of the display perch. All spectra were collected between 0540 and 1130 CST. To control for time of day and seasonal effects, readings at each lek were collected in a single measurement session, so that we were able to obtain irradiance spectra for all three light environments in only a few cases (i.e., when sunny and cloudy conditions occurred in close succession). We obtained forest shade readings at 24 primary display perches,

small gap readings at 15 primary display perches, and cloudy readings at 3 primary display perches. We transformed readings into units of photon flux as described by Endler (1990) and used a mean irradiance spectrum from each of the three light environments in the visual models described below.

Visual modeling

Single–cone photoreceptors are responsible for color discrimination in birds, and most diurnal birds have four types (Cuthill et al. 2000; Hart 2001). These four cone types are characterized by the wavelengths to which they are most sensitive: long–wavelength sensitive (LWS), medium–wavelength sensitive (MWS), short–wavelength sensitive (SWS), and ultraviolet/violet sensitive (UVS/VS). The spectral sensitivity of LWS, MWS, and SWS photoreceptors is highly conserved in birds, whereas the spectral sensitivity of UVS/VS photoreceptors peaks near 370 nm (UVS) in most passerines or near 410 nm (VS) in most non–passerines (Hart 2001).

The color of an object can be represented by a point in perceptual color space whose coordinate axes represent the quantum catches of cone photoreceptors (Goldsmith 1990). For birds, this perceptual space can be likened to a tetrahedron, with each of the four photoreceptor types located at a vertex of this tetrahedron (Burkhardt 1989; Goldsmith 1990). Variation in how two different colors are perceived can be approximated by calculating Euclidean distances between two points in this tetrachromatic color space (Goldsmith 1990). However, distances between points in such a color space do not correspond directly to perceived differences in color because these distances must exceed a certain threshold to be distinguishable, and this threshold

depends on noise that originates in photoreceptors and at further stages of neural processing (Vorobyev and Osorio 1998; Vorobyev et al. 1998). Vorobyev and colleagues have developed receptor–noise limited color space models that take into account visual sensitivities, transmission of the ocular media, light environment, visual background, and receptor noise, and these models agree well with behavioral data in a variety of taxa (Vorobyev and Osorio 1998; Vorobyev et al. 1998; Vorobyev et al. 2001; Osorio and Vorobyev 2005).

We implemented an avian version of this color space model to calculate how different colored plumage patches would be perceived by manakins. All equations follow Vorobyev et al. (1998) and further details are provided in Doucet et al. (2006b). Spectral sensitivities have not been measured in manakins; however, most passerine birds have UVS photoreceptors (Hart 2001), and long–tailed manakins have SWS1 opsin sequences consistent with ultraviolet sensitivity (S. M. Doucet, H. L. Mays, and G. E. Hill, unpubl. data). We therefore used spectral sensitivity data from the blue tit, (*Parus caeruleus*) a species with UVS cones (Hart et al. 2000; Hart 2001), to estimate photoreceptor quantum catches in manakins. These data include the effects of colored oil droplets, and photoreceptor sensitivities are calculated based on best–fitted pigment templates (Govardovskii et al. 2000; Hart et al. 2000; Hart 2001).

For all manakin plumage patches, we calculated photoreceptor quantum catch (Q) as a proportion of total quantum catch for each of the four types of avian photoreceptors using the following equation:

$$Q_i = \frac{\int_{\lambda} R_i(\lambda) S(\lambda) I(\lambda) O(\lambda) d\lambda}{\int_{\lambda} R_i(\lambda) d\lambda} \quad (1)$$

where λ represents wavelength, $R_i(\lambda)$ is the spectral sensitivity of receptor type i, $S(\lambda)$ is the reflectance of the color patch, $I(\lambda)$ is the irradiance of the light environment, and $O(\lambda)$ is the transmission of the ocular media. Data for spectral sensitivities, irradiance, and ocular transmission were normalized to one and all data spanned the range from 300 nm to 700 nm. Using equation 1, we calculated receptor quantum catches for each single cone type (UVS, SWS, MWS, LWS). Photoreceptors undergo chromatic adaptation to pre–exposed or surrounding background stimuli, and we accounted for this by normalizing the photoreceptor quantum catches of plumage patches to the photoreceptor quantum catches of the adapting background using the von Kries scaling algorithm (Wyszecki and Stiles 1982):

$$q_i = k_i Q_i \tag{2}$$

where the scaling factor, k_i, is defined as:

$$k_i = 1/\frac{\int_\lambda R_i(\lambda)S(\lambda)I(\lambda)O(\lambda)d\lambda}{\int_\lambda R_i(\lambda)d\lambda} \tag{3}$$

where $S(\lambda)$ is the reflectance spectrum of the background. According to Fechner's law, the perceived magnitude of a visual stimulus is proportional to the physical magnitude of that stimulus (Wyszecki and Stiles 1982). the receptor signal (f_i) is therefore proportional to the normalized receptor quantum catch (q_i) and can be calculated as follows:

$$f_i = \ln(q_i) \tag{4}$$

Using equation 4, we calculated receptor signals for each of the four avian cone types. Color perception is achieved by comparing receptor signals across different

receptor types. Similarly, perceived differences in color between two objects can be determined by comparing differences in receptor signals across different receptor types. For each receptor type, the difference in receptor signals between two colored patches will equal Δf_i. For an avian tetrachromat, we can calculate the discriminability between two colored patches using the following equation:

$$\Delta S^2 = (\omega_1\omega_2)^2(\Delta f_4-\Delta f_3)^2+(\omega_1\omega_3)^2(\Delta f_4-\Delta f_2)^2+(\omega_1\omega_4)^2(\Delta f_3-\Delta f_2)^2+$$
$$(\omega_2\omega_3)^2(\Delta f_4-\Delta f_1)^2+(\omega_2\omega_4)^2(\Delta f_3-\Delta f_1)^2+(\omega_3\omega_4)^2(\Delta f_2-\Delta f_1)^2/ \quad (5)$$
$$[(\omega_1\omega_2\omega_3)^2+(\omega_1\omega_2\omega_4)^2+(\omega_1\omega_3\omega_4)^2+(\omega_2\omega_3\omega_4)^2]$$

where ΔS is the distance in tetrachromatic perceptual color space, Δf_i is the difference in receptor signals at each of the four avian receptor types (UVS, SWS, MWS, LWS), and ω_i is the noise–to–signal ratio (Weber fraction). Under bright viewing conditions, the Weber fraction can be modeled as follows:

$$\omega_i = \nu_i / \sqrt{\eta_i} \quad (6)$$

where ν is the noise–to–signal ratio in a single photoreceptor of type i and η is a scaling factor that accounts for the relative number of photoreceptors of type i. We used data from the red–billed leiothrix, *Leiothrix lutea*, (Maier 1992) to estimate noise–to–signal ratios and data from the blue tit to estimate the relative abundance of receptor types (Hart et al. 2000). ΔS can be used to measure the distance in avian perceptual color space between any two colors.

Quantifying chromatic contrast

Calculated values of ΔS quantify differences in color and not differences in brightness, so we refer to distances in perceptual color space (ΔS) as chromatic contrast.

We calculated two measures of chromatic contrast. The degree of contrast against the background influences long–distance conspicuousness – the greater the contrast, the more perceptible the signal will be from a distance (Endler 1990; Endler and Théry 1996; Heindl and Winkler 2003a). As a measure of long–distance conspicuousness, we measured the distance in perceptual color space (ΔS) between manakin plumage patches and different visual backgrounds (chromatic contrast against the background). The degree of contrast between different body regions influences short–distance conspicuousness. From a close distance, plumage patches that contrast against each other will be highly conspicuous. Beyond a certain distance, however, individual elements within a color pattern cannot be resolved and their spectral properties are summed together by the eye (Endler 1978; Heindl and Winkler 2003a). As a measure of short–distance conspicuousness, we calculated distances in perceptual color space (ΔS) between all possible combinations of plumage patches for each individual (for a total of 10 combinations) and then averaged these to obtain a mean value of chromatic contrast between body regions for each bird.

Quantifying achromatic contrast

Because variation in brightness also influences the conspicuousness of signals, we calculated measures of brightness contrast (hereafter achromatic contrast) to correspond to the measures of chromatic contrast described above. In birds, double cones are thought to be used for achromatic signal detection (Cuthill et al. 2000; Hart 2001). We therefore calculated receptor signals for double cones (f_D) using the formulae described above and spectral sensitivity data from blue tit double cones (Hart et al. 2000). We calculated f_D

directly from Q_D. Receptor noise is not known for double cones, and we therefore estimated the perceived brightness of a color patch as f_D and the perceived difference in brightness between any two patches (achromatic contrast) as Δf_D. Because there is only one type of double cone, receptor noise would be the same for all comparisons and would only affect absolute and not relative differences in receptor signals (Stuart–Fox et al. 2003); Δf_D should therefore serve as a good approximation of differences in perceived brightness. High achromatic contrast can result from the reflection of more light than the patch of comparison or less light than the patch of comparison, so we used absolute values in our analyses. As described above for chromatic contrast, we measured achromatic contrast against the background as a measure of long–distance conspicuousness and achromatic contrast between body regions as a measure of short–distance conspicuousness.

We used these models to compare plumage conspicuousness in three light environments (forest shade, small gap, or cloudy) and against two visual backgrounds (green leaves or brownish bark). We focused on these two visual backgrounds as they are representative of the background from the perspective of an observer on the display perch (male or female) or an observer located some distance away but at the same height in the forest (potential predator). Moreover, the reflectance spectra of the brownish leaf litter, display perches, and bark were all of similar shape (see below). The two backgrounds we modeled should therefore provide a good approximation of conspicuousness for a variety of viewing geometries.

Light environments and display perch location

To determine whether light environment influences display perch location in long–tailed manakins, we compared light environments at primary display perches with light environments at other locations in the forest understory. For each of 24 leks, we used the methods described above (see *Available light environments*) to measure the color of ambient light at each of four sites: (1) the primary display perch, (2) the secondary display perch (when present), (3) a control vine that resembled the primary display perch in physical characteristics but was not used as a display perch, and (4) a site 10m from the primary display perch in a pre–determined, arbitrary compass direction. For the first three sites we collected irradiance readings at the height of the perch and at the fourth site we collected readings from 65 cm above the ground (a height typical of display perches). We purposely chose control perches that appeared to be of similar dimensions to typical display perches, as we sought to determine whether differences in light environment precluded their use as display perches. We noted the light environment at the time the readings were taken and collected readings from multiple light environments at each perch when possible. To control for time of day and seasonal effects, we collected readings at each lek in a single measurement session and used pairwise comparisons within leks in our analyses.

Perch characteristics

To determine whether long–tailed manakins might choose display locations based on features other than light environment, we measured a number of physical characteristics of primary display perches, secondary display perches, and control perches

(measurement sites 1–3 above) for a subset of leks (n = 18). We measured the height of the perch, the length of the perch, the diameter of the perch, and the distance to the nearest vegetation on both sides of the perch. We averaged these last two measurements to obtain a mean value of distance to the nearest vegetation.

Light environments and timing of displays

We collected behavioral observations of male advertisement rate and display activity at each of 12 leks. Observations were recorded from blinds or concealed locations 8–10 m from primary display perches. Manakins did not display in rainy weather, and we therefore terminated any ongoing observation at the first sign of rain. Observation sessions ranged in duration from 55 to 300 minutes, and each lek was observed for a total of 340 ± 42.9 minutes (mean ± SE). During observations, we recorded all vocalizations and displays performed by male manakins in five–minute blocks. For the purposes of this study, we focused on "toledo" vocalizations, as these are advertisement duets sung by dominant males to attract females to the lek (McDonald 1989b; Trainer and McDonald 1993). We separated instances of attendance at the display perch into "displays" (two dominant males performing behavioral displays in the presence of one or more females), and "practice dances" (one or several males, dominant or subordinate, performing behavioral displays in the absence of females). Female presence was either noted directly or, when females could not be seen due to obstructing vegetation, implied by a combination of the utterance of "owng" vocalizations (Trainer and McDonald 1993) and the distinct sequence and rhythm of vocalizations and behaviors that precede and accompany displays directed at females (McDonald 1989a;

McDonald 1989b; Trainer and McDonald 1993). If the presence of a female could not be implied by both of these criteria, or if displays took place entirely out of sight at secondary or tertiary display perches, they were excluded from our analyses. We recorded the light environment throughout observation bouts as sunny (forest shade) or cloudy. For any displays performed in sunny conditions, we also noted whether a small gap was illuminating part of the display perch. All observations were collected between June 13 and July 11, in 2003 and 2004 (i.e., during the rainy season). Because we did not encounter all weather conditions at all 12 leks, our sample size was too small to allow for paired analyses. We therefore compared advertisement and display rates in different light environments using mixed models that included lek ID as a random effect.

RESULTS

Plumage and background reflectance

The black breast and flight feathers of adult male long–tailed manakins exhibited uniformly low reflectance across the bird–visible spectrum, from 300 to 700 nm (Fig. 1A). The reflectance of the blue mantle of males increased at short wavelengths, peaking in the ultraviolet, and decreased at longer wavelengths (Fig. 1A). The red crown coloration of males exhibited uniformly low reflectance at middle wavelengths, with a subtle increase in reflectance at short wavelengths and a steep increase at long wavelengths (Fig. 1A). In females, reflectance spectra for different body regions, all of which were olive–green in color, were of similar shape, increasing at short wavelengths, decreasing between 400 and 500 nm, and increasing to a plateau at longer wavelengths (Fig. 1B).

The green leaves of saplings surrounding manakin display perches exhibited a peak in reflectance between 500 and 600 nm (Fig. 2A). The brownish leaf litter below the perches, the bark of nearby trees, and the bark of display perches exhibited reflectance spectra of similar shape, with reflectance increasing steadily with wavelength across the bird–visible range (Fig. 2A, B).

We used principal components analysis (PCA) to summarize information from reflectance spectra (e.g., Endler 1990; Cuthill et al. 1999; Heindl and Winkler 2003a; Montgomerie 2006). We first reduced the number of variables represented by each spectrum by calculating average reflectance values in 10 nm–sized bins from 300 to 700 nm, resulting in 40 reflectance values (variables) per spectrum. We included as observations reflectance spectra from the five body regions of each manakin, as well as all spectra collected from leaves, leaf litter, bark, and display perches. This PCA produced three principal component variables with eigenvalues ≥ 1, together accounting for more than 99% of the variation in reflectance. Component loadings for the first principal component (reflectance PC1), which explained 77 % of the variation in reflectance, were moderate and positive from 300 to 600 nm (Fig. 3). Component loadings for the second principal component (reflectance PC2), which explained 19% of the variation in reflectance, were high and positive from 600 nm to 700 nm (Fig. 3). Component loadings for the third principal component, which explained 4% of the variation in reflectance, were moderate and positive from 300 to 450 nm and from 650 to 700 nm, whereas they were high and negative from 500 to 625 nm (Fig. 3).

Reflectance PC scores differed significantly between males, females, and the visual background (reflectance PC1: $F = 32.4$, $df = 2, 168$, $P = 0.04$; reflectance PC2: $F =$

5.86, df = 2, 168, P = 0.003; reflectance PC3: F = 301.5, df = 2, 168, P <0.0001). Among manakins, there were significant differences based on sex, body region, and the interaction between sex and body region for reflectance PC1 scores (whole model: F = 769.2, df = 9, 599, P < 0.0001; sex: F = 44.0, df = 1, 607, P < 0.0001; body region: F = 802.9, df = 4, 604, P < 0.0001; sex*body region: F = 873.2, df = 4, 604, P < 0.0001), reflectance PC2 scores (whole model: F = 1193.0, df = 9, 599, P <0.0001; sex: F = 34.2, df = 1, 607, P < 0.0001; body region: F = 1047.2, df = 4, 604, P < 0.0001; sex*body region: F = 1536.7, df = 4, 604, P < 0.0001) and reflectance PC3 scores (whole model: F = 432.1, df = 9, 599, P < 0.0001; sex: F = 3308.1, df = 1, 608, P < 0.0001 ; body region: F = 61.4, df = 4, 604, P < 0.0001; sex*body region: F = 54.4, df = 4, 604, P < 0.0001).

These reflectance PC scores can be used to represent reflectance information in a three–dimensional color space that is independent of the visual system (Endler 1990). Male plumage patterns generally encompassed a larger portion of this color space than female patterns and background elements (Fig. 4). In particular, the blue mantle and red crown of males occupied unique areas of the color space, whereas female plumage patterns overlap almost entirely with background elements (Fig. 4). Indeed, the 95% density ellipses of female reflectance PC scores are completely encompassed within the 95% density ellipses of background elements (Fig. 4); that is, variation in female plumage reflectance is a small subset of the variation in background reflectance. A discriminant analysis based on these PC scores revealed significant differentiation of males, females, and background elements (F = 203.9, df = 6, 1438, P < 0.0001). However, whereas 99% of male values were correctly classified as male, 33% of female

values were misclassified as background elements, and 49% of background elements were misclassified as female.

Light environments

In the forest understory at our study site, there were three light environments available for display: forest shade, small gaps, and cloudy (Endler 1993a). We measured each of these light environments at long–tailed manakin primary display perches. Forest shade light was greenish in color, as revealed by relatively high irradiance at middle wavelengths and relatively low irradiance at short and long wavelengths (Fig. 5A). By contrast, small gap light exhibited a nearly inverse pattern, with relatively high irradiance at short and long wavelengths and relatively low irradiance at middle wavelengths (Fig. 5B). Cloudy light exhibited low irradiance at ultraviolet wavelengths and a more even distribution in irradiance across the remainder of the spectrum (Fig. 5C).

Visual modeling

Effects of sex and body region

In all combinations of light environment (forest shade, small gap, or cloudy) and visual background (leaves or bark), there were significant effects of sex, body region, and the interaction between sex and body region on both chromatic (Fig. 6A,Table 1) and achromatic (Fig. 6B, Table 2) contrast against the background, with males exhibiting more contrast against the background than females in all cases. Similarly, there were significant sex differences in chromatic contrast between body regions (Fig. 7A) and achromatic contrast between body regions (Fig. 7B) in all three light environments (Table

3), with males exhibiting more contrast between body regions in all cases. Thus, male plumage coloration was more conspicuous than female plumage coloration from both long and short distances.

Effects of light environment and the visual background

There was a significant influence of both the light environment and the visual background on the degree of chromatic plumage contrast against the background in male (Fig. 8A, Table 4) and female (Fig. 8B, Table 4) long–tailed manakins. Among males, chromatic plumage contrast against the background was highest in small gaps, intermediate in cloudy conditions, and lowest in forest shade, for both types of background (Fig. 8A). A similar pattern was found in females (Fig. 8B). Despite the significant influence of light environment, however, values of chromatic contrast against the background were more strongly influenced by the visual background (Fig. 8, Table 5). Among males, chromatic plumage contrast against the background was highest when the background was composed of bark (Fig. 8A), whereas among females, chromatic plumage contrast against the background was highest when the background was composed of leaves (Fig. 8B).

There was a significant influence of both light environment and the visual background on achromatic plumage contrast against the background in both males (Fig. 9A, Table 4) and females (Fig. 9B, Table 4). Among males, achromatic plumage contrast against the background was highest in cloudy conditions, intermediate in forest shade, and lowest in small gaps, for both types of background. Moreover, achromatic plumage contrast against the background was significantly higher when the background was

composed of leaves than bark (Fig. 9A, Table 5). Among females, achromatic plumage contrast against the background was highest in cloudy conditions, intermediate in forest shade, and lowest in small gaps when the background was composed of leaves (Fig. 9B, Table 4). However, when the background was composed of bark, achromatic plumage contrast against the background was higher in small gaps than in forest shade or cloudy conditions, and there were no significant differences in contrast against the background between the latter two light environments (Fig. 9B, Table 4). When illuminated by forest shade or cloudy conditions, females exhibited greater achromatic plumage contrast against the background when the background was composed of leaves (Fig. 9B, Table 5). By contrast, where illuminated by small gaps, females exhibited greater achromatic plumage contrast against the background when that background was composed of bark (Fig. 9B, Table 5).

The degree of chromatic and achromatic contrast between body regions is also influenced by variation in the light environment in male and female long–tailed manakins (Fig. 7A,B, Table 6). Among males, the degree of chromatic and achromatic contrast between body regions was highest in small gaps, intermediate in forest shade, and lowest in cloudy conditions (Fig. 7A,B). Among females, the degree of chromatic and achromatic contrast between body regions was highest in small gaps, intermediate in cloudy conditions, and lowest in forest shade (Fig. 7A,B).

Display location and light environment

We compared irradiance readings collected at four locations for each of 24 leks: the primary display perch, the secondary display perch, a nearby control perch, and a

location 10 m from the primary display perch in an arbitrary compass direction. We used PCA to summarize information from irradiance spectra into a few orthogonal variables as described above for reflectance spectra. This PCA produced 3 principal components with eigenvalues ≥ 1. Component loadings for the first principal component (irradiance PC1), which explained 82% of the variation in irradiance, were moderate and positive from 300 to 700 nm (Fig. 10). Component loadings for the second principal component (irradiance PC2), which explained 14% of the variation in irradiance, were high and positive 300 to 400 nm and moderate and negative from 500 to 600 nm (Fig. 10). Component loadings from the third principal component (irradiance PC3), which explained 3% of the variation in irradiance, were high and positive from 300 to 350 and from 500 to 600 nm and high and negative from 350 to 400 nm (Fig. 10).

In paired analyses, there were no significant differences in irradiance PC scores between readings collected in forest shade light environments at primary display perches versus those collected at secondary perches, nearby control vines not used for display, or at arbitrary locations 10 m from the primary perch (Table 7). Similarly, there were no significant differences in irradiance PC scores between readings collected in small gap light environments at primary display perches versus those collected at secondary perches, nearby control vines not used for display, or at arbitrary locations 10 m from the primary perch (Table 7). Our sample size was too small to allow for comparisons in cloudy light environments.

We also compared the physical characteristics of primary perches, secondary perches, and control vines. There were no significant differences in perch height (Fig. 12A; $F = 0.09$, $df = 2, 46$, $P = 0.91$). There were significances in perch length (Fig. 12B;

F = 2.53, df = 2, 46, P = 0.04), although Tukey–Kramer tests did not reveal significant differences between any particular pair of perches (P > 0.05). There were significant differences in perch diameter among the three types of perch (Fig. 12C; F = 4.04, df = 2, 46, P = 0.02); primary and control perches were significantly thicker than secondary perches (Tukey–Kramer tests, P < 0.05). Finally, there were significant differences in the distance to the nearest vegetation (Fig. 12D; F = 4.91, df = 2, 46, P = 0.01); the nearest vegetation was significantly farther away from primary display perches than from secondary and control perches (Tukey–Kramer tests, P < 0.05).

Timing of display and light environment

We collected a total of 3307 minutes of observation in forest shade conditions and 775 minutes of observation in cloudy conditions. We did not continuously monitor the presence or absence of small gaps on display perches, as these were relatively uncommon and lasted only for a few seconds. However, we did note whether a small gap illuminated part of the display perch during displays directed at females or practice dances. In total, 85% of displays directed at females were performed in forest shade, 12 % in cloudy conditions, and 3% when the perch was partly illuminated by a small gap. By comparison, 64% of practice dances were performed in forest shade, 34% in cloudy conditions, and 2% when the perch was partly illuminated by a small gap. Birds made no attempt to move into or avoid small gaps when they were present and simply displayed in the usual manner. There was no significant difference in the rates of toledo calls given in forest shade (2.15 ± 0.58 toledos/min) versus cloudy (1.64 ± 0.71 toledos/min) light environments (whole model: F = 0.81, df = 12, 14, P = 0.63; light environment: F = 0.57,

df = 1, 14, P = 0.46; lek: F = 0.85, df = 11,14, P = 0.60). There was no significant difference in the rates of practice dances performed in forest shade (0.76 ± 1.05 practices/hour) versus cloudy (3.88 ± 1.30 practices/hour) light environments (whole model: 1.14, df = 12, 14, P = 0.39; light environment: F = 4.92, df = 1, 14, P = 0.04; lek: F = 0.85, df = 11, 14, P = 0.60). There was no significant difference in the rates of displays directed at females in forest shade (0.48 ± 1.02 displays/hour) versus cloudy (2.22 ± 1.27 displays/hour) light environments (whole model: F = 0.54, df = 12, 14, P = 0.85; light environment: F = 0.91, df = 1, 14, P = 0.23; lek: F = 0.90, df = 11, 14, P = 0.35).

DISCUSSION

In this study, we investigated the influence of the light environment and the visual background on plumage conspicuousness in long–tailed manakins and determined what influence, if any, these factors exerted on spatial and temporal patterns of display in this species. Our first objective was to quantify the reflectance of plumage patches of male and female manakins and to compare them with the reflectance of the leaves, bark, display perches, and leaf litter constituting the visual background of manakin display areas. The black wings, tail, and breast feathers of adult males exhibited low, uniform reflectance across the bird–visible spectrum. By contrast, their sky–blue mantles and bright red crowns exhibited prominent peaks at opposite ends of the spectrum in the ultraviolet and red regions, respectively. The plumage patches of females exhibited reflectance spectra typical of olive–green plumage, with a subtle peak in reflectance in the UV and a low plateau at longer wavelengths (Dyck 1978; Doucet and Montgomerie

2003b). The reflectance spectra of green leaves surrounding manakin display perches peaked at middle wavelengths, as expected from the absorbance properties of chlorophyll (Endler 1993a; Andersson et al. 1998). The reflectance spectra of the brownish leaf litter beneath display perches, the bark of nearby trees, and the bark of the display perches themselves all exhibited similar shapes, with steadily increasing reflectance from short to long wavelengths (Endler 1993a).

We used principal components analysis (PCA) to summarize reflectance data and construct a visual color space that is independent of variation in the visual system. There were significant differences between reflectance PC scores of male and female manakins and those of background elements. Among manakins, reflectance was strongly influenced by sex, body region, and the interaction between sex and body region. Male plumage colors generally occupied larger portions of the PCA color space than female plumage colors or background elements. Indeed, the red crown and blue mantle males were unique colors in the signaling environment of manakins. By contrast, female plumage patterns occupied a small subset of the color space defined by the visual environment of manakins, overlapping with the coloration of green leaves, brownish leaf litter, and bark. Moreover, a discriminant function analysis based on reflectance PC scores correctly classified 99% of male plumage patches, but misclassified 33% of female reflectance scores as background elements and 49% of background elements as female plumage patches.

Our second objective was to document the different light environments available at manakin display sites. Long–tailed manakins performed their displays at fixed locations on display perches in the understory of evergreen forest. Light environments in

forests vary as a function of forest geometry (closed versus open canopy, small versus large canopy gaps), weather conditions (sunny versus cloudy), and time of day (crepuscular versus daylight) (Endler 1993a). Long–tailed manakin display perches were not associated with canopy gaps or forest edges, and males displayed for females after sunrise, precluding the use of crepuscular light environments (cf. Endler and Théry 1996; Doucet and Montgomerie 2003a).

During the first half of the breeding season, which coincides with the dry season, sunny conditions prevail and only two light environments are available to displaying manakins: forest shade and small gaps (Endler 1993a). Under such sunny conditions, forest shade light predominates in the shady understory of closed–canopy forests. Forest shade irradiance spectra were rich in middle wavelengths and were greenish in color, as expected since much of the radiant light is reflected from the surrounding vegetation (Endler 1993a; Endler and Théry 1996; Heindl and Winkler 2003a). Forest shade light environments are usually interspersed with small gaps (or sun flecks), where the sun penetrates directly to the understory through small interruptions in the canopy. Small gap light environments are typically dynamic and unpredictable (Heindl and Winkler 2003a). At our study site, any particular small gap usually lasted for very brief periods only, especially during the dry season, when strong winds persistently moved the leaves in the forest canopy. Small gap light environments are usually reddish in color because much of the light comes directly from the sun (Endler 1993a; Endler and Théry 1996; Heindl and Winkler 2003a), whereas our small gap measurements exhibited two irradiance maxima, one at short UV/blue wavelengths, and a second at long reddish wavelengths. This difference may have resulted from our measurement geometry. All of our irradiance

readings were collected with the probe oriented directly skyward. However, in the morning, when we collected our measurements, the sunlight of small gaps penetrated the forest obliquely and the solar disk was usually partially obstructed by vegetation, with the combined effect of a reduction in the relative contribution of direct sunlight and an increase the relative contributions of blue sky and vegetation to the total irradiance (Endler 1993a). Since the direct sunlight and blue sky are much brighter than the vegetation (Endler 1993a), such conditions could produce a light environment rich in blue and red wavelengths and poor in green wavelengths.

In the latter part of the breeding season, which coincides with the rainy season, intermittent sunny, cloudy, and rainy conditions make a third light environment available for display: cloudy (Endler 1993a). As in other studies, irradiance measurements collected in cloudy conditions were relatively poor in UV light and otherwise whitish in appearance, as revealed by a generally even distribution of light across the remainder spectrum and as expected from the light–scattering properties of clouds (Endler 1993a; Endler and Théry 1996; Heindl and Winkler 2003a).

We integrated these data on plumage reflectance, background reflectance, and light environments into an avian perceptual color space model to examine the influence of the visual environment on the conspicuousness of long–tailed manakin plumage patterns. We examined the influence of two different backgrounds (green leaves and brownish bark) and three different light environments (forest shade, small gap, and cloudy). We found striking sex differences conspicuousness. In all combinations of light environment and visual background, males exhibited higher chromatic and achromatic contrast against the background than did females for all body regions. In terms of

chromatic contrast, this effect was more pronounced for the red crown and blue mantle, and in terms of achromatic contrast, this effect was more pronounced for the black breast, wings, and tail, suggesting that these different body regions emphasize different types of conspicuousness. Males also exhibited more chromatic and achromatic contrast between body regions than did females.

Three lines of evidence suggest that female long–tailed manakins are remarkably cryptic. First, female coloration is a subset of the colors constituting the visual background in the forest understory habitat of manakins. Second, based on our avian perceptual model, females exhibit low degrees of contrast against the background, a measure of long–distance conspicuousness. Third, females exhibit low degrees of contrast between body regions, a measure of short–distance conspicuousness. By these same arguments, males are relatively conspicuous, as they display unique colors not found elsewhere in their habitat, exhibit high degrees of contrast against the background, and even higher degrees of contrast between body regions. Together with recent studies that have documented similar patterns in other manakins (Endler and Théry 1996; Uy and Endler 2004; Doucet et al. 2006b), our findings highlight the opposing selection pressures on males and females. Sexual selection is thought to act particularly strongly on males in lek–mating manakins (McDonald 1993; Prum 1997; Snow 2004) and has probably played a critical role in the evolution of conspicuous male ornaments. By contrast, natural selection has likely favored the evolution and maintenance of cryptic coloration in females, even in the face of opposing pressure from genetic correlations between the sexes (Lande 1987). Selection for cryptic coloration may be particularly important in

female manakins as they are solely responsible for rearing offspring (Snow 2004) and may be particularly vulnerable during incubation and nestling care.

Our findings highlight the importance of considering the different selective pressures that act on males and females when trying to understand observed patterns of sexual dichromatism (Badyaev and Hill 2003). In many cases, sexual dichromatism is a consequence of selection on male coloration to diverge from the background (i.e., selection for conspicuousness) and selection on female coloration to match the background (i.e., selection for crypsis) rather than a consequence of selection on male coloration to diverge from female coloration. Our findings suggest that caution is warranted in the use of sexual dichromatism as an index of the intensity of sexual selection; in species where both males and females experience selection pressures for plumage coloration that contrasts against the background, the degree of sexual dichromatism will likely underestimate the intensity of social selection on either sex (Amundsen 2000; Amundsen 2002).

We found significant influences of both light environment and background coloration on the conspicuousness of male and female plumage patterns. Regardless of variation in light environment, males exhibited more chromatic contrast against the background when the background was composed of bark and more achromatic contrast against the background when the background was composed of leaves. Females exhibited more achromatic and chromatic contrast against the background when the background was composed of leaves. Females would therefore be more cryptic against a background composed mostly of bark, whereas males would be relatively conspicuous against either background, one background emphasizing achromatic (brightness) contrast, and the other

emphasizing chromatic (color) contrast. It is worth noting that background coloration had a larger influence on conspicuousness than light environment. This makes intuitive sense because in most situations, a change in light environment will influence the coloration of the target individual and of the visual background in similar ways. To date, however, research has emphasized variation in light environments whereas background coloration has often been overlooked. Our findings argue that the visual background should be given equal consideration in studies of visual conspicuousness.

The conspicuousness of plumage features varied significantly across the three primary light environments. Male chromatic plumage contrast against the background was highest in small gaps, intermediate in cloudy conditions, and lowest in forest shade whereas achromatic plumage contrast against the background was highest in cloudy conditions, intermediate in forest shade, and lowest in small gaps. Additionally, among males, chromatic and achromatic contrast between plumage regions was highest in small gaps, intermediate in forest shade, and lowest in cloudy conditions. Among females, chromatic and achromatic contrast against the background and chromatic and achromatic contrast between body regions were highest in small gaps, intermediate in cloudy conditions, and lowest in forest shade. Thus, in terms of long–distance conspicuousness, both males and females were generally most conspicuous in small gaps. In terms of short–distance conspicuousness, males were most conspicuous in small gaps and least conspicuous in cloudy conditions whereas females were most conspicuous in small gaps and least conspicuous in forest shade. If male conspicuousness is the primary selective target and the timing and location of displays are relatively unconstrained, males should preferentially display in small gaps. However, both males and females are exposed to the

same light environments on display perches, and long–tailed manakins may experience selective pressure to simultaneously minimize female conspicuousness.

Given the substantial influence of light environment on plumage conspicuousness, we wondered whether light environment might influence the location of display perches. In paired comparisons, light conditions at primary display perches did not differ from light conditions at secondary display perches, nearby control vines not used for display, or an arbitrary location 10 m from the primary display perch in either forest shade or small gap light environments. That is, light environments at manakin display perches were no different than light environments in other parts of the forest understory, implying that long–tailed manakins did not choose display perch locations on the basis of variation in the light environment. However, a number of other factors may influence the initial choice of display perch location. First, display perch location is almost certainly influenced by large–scale factors such as proximity to foraging areas and distance from other established leks (McDonald 1989b; Théry 1992). Second, perches must be located in areas that allow males to perform butterfly display flights, and must therefore be relatively unobstructed by vegetation. Indeed, although our control sites (nearby vines not used for display) resembled primary display perches in physical characteristics, potentially obstructing vegetation was located significantly farther away from primary perches than from control perches. The fact that males purposely remove obstructing leaves (D. B. McDonald, unpubl. data), as has been shown in other species (Snow 1963; Endler and Théry 1996), anecdotally supports this hypothesis. Third, once selected, display perches are commonly used for up to 10 years and, in some cases, much longer (D. B. McDonald, unpubl. data). Although such extended use of display perches likely

facilitates long–term lek fidelity by females (McDonald and Potts 1994), it is probably longer than most non–random subsets of forest light environments, for example large canopy gaps, are expected to last. Finally, the location of display perches in non–random subsets of the light environment would make the entire display area more conspicuous to predators at a time when both males and females are likely to be less vigilant, their attention focused on performing or observing displays (Trail 1987).

Finally, we investigated whether variation in light environment influences the timing of display in long–tailed manakins. In the first half of the breeding season, both forest shade and small gap environments were available for display and in the latter part of the breeding season cloudy light environments became available as well. In comparisons of cloudy versus forest shade light environments, there were no significant differences in the rates of advertisement duets sung by males, the rates displays performed for females by dominant males, or the rates practice dances performed by males. Small gaps were ephemeral in nature, lasting for very brief periods only, often only a few seconds at a time. Even when a small gap was illuminating part of the display perch, males did not appear to move into or avoid these gaps. Indeed, the dynamic nature of the dual–male cooperative display – where each male must coordinate leapfrog and butterfly displays with his partner and maneuver around one or more females on the display perch – may preclude males from altering their display behavior in relation to small gaps. Thus, long–tailed manakins do not appear to adjust the timing of their displays to take advantage of particular subsets of the light environment. Instead, our data suggest that long–tailed manakins perform their displays in proportion to the availability of different light environments: 85% of displays were performed in forest shade, 12% in

cloudy conditions, and 3% in small gaps. Had we performed behavioral during the dry season, displays would surely be even more skewed toward forest shade light conditions.

The perceptual environment can influence visual signals in animals in two ways. First, the visual environment can dictate what sort of coloration should evolve for an individual to achieve either crypsis or conspicuousness. Alternatively, behaviors can evolve that allow animals to adjust the timing or location of their displays to take advantage of particular light environments. While the latter does not appear to apply in long–tailed manakins, the former may still hold true. According to our visual models, displays in forest shade may offer a partial resolution to the conflict of selection for conspicuous male coloration and cryptic female coloration. In forest shade, female conspicuousness is minimized at both short and long distances and male conspicuousness is intermediate at short distances and intermediate or least conspicuous at long distances. In comparison with forest shade, small gaps increase the long– and short–distance conspicuousness of both males and females, and cloudy light environments increase the conspicuousness of females while reducing that of males. Displays in forest shade thereby allow males to optimize their short–distance conspicuousness while minimizing their long–distance conspicuousness and the overall conspicuousness of females (Endler and Théry 1996; Heindl and Winkler 2003a). In addition, among males, contrast between body regions is greater than contrast against the background in all combinations of light environment and backgrounds, lending support to the idea that short–distance conspicuousness is favored over long–distance conspicuousness. While these interpretations remain speculative, a recent comparative analysis across the Pipridae revealed that in forest shade light environments and against a green vegetation

background, male plumage patterns are relatively conspicuous whereas female plumage patterns are relatively cryptic, suggesting that the visual environment has had a general influence on the evolution of coloration patterns in these birds (Doucet et al. 2006b).

Several species of lek–mating birds, including many manakins, have been shown to display in non–random subsets of the light environment that enhance the conspicuousness of their color patterns (Endler and Théry 1996; Heindl and Winkler 2003b; Heindl and Winkler 2003a; Uy and Endler 2004). Interestingly, long–tailed manakins differ from all other species investigated in two respects. First, long–tailed manakins, along with other *Chiroxiphia* manakins (Snow 1963; Foster 1981), are the only species that perform obligate cooperative displays (Snow 2004). As described above, these affiliations may impose constraints on the timing and location of displays in both the short term (coordination on the display perch) and long term (female lek fidelity). Second, the distribution of long–tailed manakins does not overlap with any other manakin, whereas other species investigated are sympatric with multiple other manakins (Théry 1992; Endler and Théry 1996; Heindl and Winkler 2003b; Heindl and Winkler 2003a). Because most manakins follow a lek–based mating system (Snow 2004), there may be some selective pressure for manakins to minimize potential interference from other species by modifying the timing or location of their displays. Red–capped manakins (*Pipra mentalis*), for example, are known to display at greater heights in the forest where they overlap with blue–crowned manakins (*Lepidothrix coronata*), a species that displays in the understory (Stiles and Skutch 1989). Selective pressure to minimize physical, vocal, or visual interference from other species may therefore drive the spatial and/or temporal segregation of displays among sympatric manakins species. Of course, any

change in the time of day, time of year, or location of manakin displays would likely also result in a change of light environment (Endler 1993a; Heindl and Winkler 2003b). Thus, selective pressure for the temporal and/or spatial segregation of displays may have been sufficient to initiate microhabitat display preferences in sympatric species of manakins, or may have acted as a reinforcing mechanism, strengthening pre–existing preferences for light environments that optimized plumage conspicuousness. A comparative analysis of coloration patterns and light environments used for display in sympatric versus allopatric manakins would provide a compelling test of these hypotheses.

In conclusion, our study reveals an important influence of the light environment and visual background on the conspicuousness of male plumage coloration and the crypsis of female plumage coloration in long–tailed manakins. Our findings contribute to a growing body of evidence suggesting that the visual environment has and an important influence on the evolution and diversity of coloration patterns found in animals.

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Table 1. The influence of sex, body region, and sex by body interactions on chromatic (color) plumage contrast against the background in long–tailed manakins for two different backgrounds (leaves and bark) and three different light environments (forest shade, small gap, and cloudy).

	Leaves Background			Bark Background		
	F	**df**	**P**	**F**	**df**	**P**
Forest shade						
Whole model	1623.8	9, 599	<0.0001	1230.1	9,599	<0.0001
Sex	2003.3	1, 507	<0.0001	3366.8	1, 507	<0.0001
Body region	1657.3	4, 504	<0.0001	906.73	4, 504	<0.0001
Sex* Body region	1391.0	4, 504	<0.0001	954.07	4, 504	<0.0001
Small gap						
Whole model	1633.1	9, 599	<0.0001	1228.6	9, 599	<0.0001
Sex	1952.7	1, 507	<0.0001	3396.1	1, 507	<0.0001
Body region	1684.4	4, 504	<0.0001	900.95	4, 504	<0.0001
Sex* Body region	1396.5	4, 504	<0.0001	949.55	4, 504	<0.0001
Cloudy						
Whole model	1604.3	9, 599	<0.0001	1213.5	9, 599	<0.0001
Sex	1986.2	1, 507	<0.0001	3331.1	1, 507	<0.0001
Body region	1640.1	4, 504	<0.0001	894.84	4, 504	<0.0001
Sex* Body region	1370.1	4, 504	<0.0001	938.46	4, 504	<0.0001

Table 2. The influence of sex, body region, and sex by body interactions on achromatic (brightness) plumage contrast against the background in long–tailed manakins for two different backgrounds (leaves and bark) and three different light environments (forest shade, small gap, and cloudy).

	Leaves Background			**Bark Background**		
	F	**df**	**P**	**F**	**df**	**P**
Forest shade						
Whole model	328.1	9, 599	<0.0001	319.5	9,599	<0.0001
Sex	2192.6	1, 507	<0.0001	2168.7	1, 507	<0.0001
Body region	77.55	4, 504	<0.0001	68.68	4, 504	<0.0001
Sex* Body region	109.3	4, 504	<0.0001	105.4	4, 504	<0.0001
Small gap						
Whole model	332.2	9, 599	<0.0001	333.5	9, 599	<0.0001
Sex	1883.5	1, 507	<0.0001	1888.4	1, 507	<0.0001
Body region	96.88	4, 504	<0.0001	97.75	4, 504	<0.0001
Sex* Body region	174.24	4, 504	<0.0001	174.9	4, 504	<0.0001
Cloudy						
Whole model	321.2	9, 599	<0.0001	309.3	9, 599	<0.0001
Sex	2285.6	1, 507	<0.0001	2252.6	1, 507	<0.0001
Body region	67.5	4, 504	<0.0001	53.21	4, 504	<0.0001
Sex* Body region	81.87	4, 504	<0.0001	78.43	4, 504	<0.0001

Table 3. Sex difference in chromatic and achromatic contrast between body regions in long–tailed manakins for three different light environments (forest shade, small gap, and cloudy).

	F	df	P
Chromatic			
Forest shade	2906.2	1, 119	<0.0001
Small gap	2953.6	1, 119	<0.0001
Cloudy	3012.6	1, 119	<0.0001
Achromatic			
Forest shade	784.6	1, 119	<0.0001
Small gap	867.9	1, 119	<0.0001
Cloudy	743.1	1, 119	<0.0001

Table 4. Paired comparisons of chromatic and achromatic plumage contrast against the background for long–tailed manakins in different light environments. Data were analyzed separately within each sex and against each type of background.

	Forest shade vs. small gap			Forest shade vs. cloudy			Cloudy vs. small gap		
	t	**n**	**P**	**t**	**n**	**P**	**t**	**n**	**P**
Chromatic									
Females									
Leaves	–33.1	59	<0.0001	–21.0	59	<0.0001	–33.6	59	<0.0001
Bark	–89.3	59	<0.0001	–89.4	59	<0.0001	–70.5	59	<0.0001
Males									
Leaves	–27.1	62	<0.0001	–18.5	62	<0.0001	–23.5	62	<0.0001
Bark	–58.4	62	<0.0001	–33.5	62	<0.0001	–39.9	62	<0.0001
Achromatic									
Females									
Leaves	6.74	59	<0.0001	–14.32	59	<0.0001	9.11	59	<0.0001
Bark	–7.38	59	<0.0001	–1.31	59	0.20	–4.48	59	<0.0001
Males									
Leaves	44.6	62	<0.0001	–37.1	62	<0.0001	42.8	62	<0.0001
Bark	35.7	62	<0.0001	–27.03	62	<0.0001	33.5	62	<0.0001

Table 5. Paired comparisons of chromatic and achromatic plumage contrast against the background for long–tailed manakins in viewed against different backgrounds. Data were analyzed separately within each sex and light environment.

	Leaves vs. bark		
	t	**n**	**P**
Chromatic			
Females			
Forest shade	7.83	59	<0.0001
Small gap	6.72	59	<0.0001
Cloudy	7.33	59	<0.0001
Males			
Forest shade	–28.7	62	<0.0001
Small gap	–34.37	62	<0.0001
Cloudy	–31.1	62	<0.0001
Achromatic			
Females			
Forest shade	7.46	59	<0.0001
Small gap	–8.10	59	<0.0001
Cloudy	7.70	59	<0.0001
Males			
Forest shade	92.0	62	<0.0001
Small gap	–92.0	62	<0.0001
Cloudy	92.0	62	<0.0001

Table 6. Paired comparisons of chromatic and achromatic plumage contrast between body regions for long–tailed manakins in different light environments. Data were analyzed separately within each sex.

	Forest shade vs. small gap			Forest shade vs. cloudy			Cloudy vs. small gap		
	t	**n**	**P**	**t**	**n**	**P**	**t**	**n**	**P**
Chromatic									
Females	–20.8	59	<0.0001	24.5	59	<0.0001	–13.8	59	<0.0001
Males	–6.1	62	<0.0001	3.94	62	0.0002	–31.4	62	<0.0001
Achromatic									
Females	–13.9	59	<0.0001	–16.3	59	0.20	7.29	59	<0.0001
Males	–21.5	62	<0.0001	10.6	62	<0.0001	–18.5	62	<0.0001

Table 7. Paired comparisons of irradiance PC scores at primary display perches of long–tailed manakins versus secondary display perches, nearby control perches not used for display, and an arbitrary location 10 m from the primary display perch. Data were analyzed separately within light environment and PC score.

	Forest shade			**Small gap**		
	t	n	P	t	n	P
Irradiance PC1						
primary vs. secondary	0.11	15	0.91	0.73	11	0.48
primary vs. control	0.48	24	0.63	–1.15	14	0.27
primary vs. arbitrary	2.05	24	0.06	0.33	14	0.74
Irradiance PC2						
primary vs. secondary	–0.55	15	0.59	–0.44	11	0.67
primary vs. control	–0.45	24	0.66	–0.48	14	0.64
primary vs. arbitrary	–0.21	24	0.84	–0.27	14	0.79
Irradiance PC3						
primary vs. secondary	0.69	15	0.50	–0.53	11	0.61
primary vs. control	–0.29	24	0.77	–0.71	14	0.49
primary vs. arbitrary	–0.53	24	0.60	–0.82	14	0.42

FIGURE CAPTIONS

Figure 1. Mean reflectance spectra of the mantle and crown (A) and of the breast, tail, and wings (B), of adult male long–tailed manakins (n = 62). Mean reflectance spectra of the mantle and crown (C) and of the breast, tail, and wings (D) of female (n = 59) long–tailed manakins. Vertical bars indicate standard errors. Note differences in scale of y–axes.

Figure 2. (A) Mean reflectance spectra of the green leaves (n = 56) of saplings surrounding long–tailed manakin display perches and of the leaf litter (n = 28) beneath manakin display perches. (B) Mean reflectance spectra of primary display perches (n = 14) and the bark of trees near primary display perches (n = 18). Vertical bars indicate standard errors.

Figure 3. Component loadings for the first three principal components (PC1, PC2, and PC3) of a principal components analysis (PCA) of reflectance spectra from plumage patches of male and female long–tailed manakins (Fig. 1) and of the bark and leaves constituting the visual background of long–tailed manakin display sites (Fig.2).

Figure 4. Scatterplots of reflectance PC scores representing the color space of male (blue) and female (red) manakin plumage coloration and components of the visual background (green). The different body regions of manakins are represented by solid circles (crown), open circles (mantle), solid triangles (tail), solid diamonds (wing), and solid squares (breast). The different components of the visual background are represented

by solid squares (bark), solid circles (leaves), open circles (leaf litter), and solid triangles (display perches).

Figure 5. Mean irradiance spectra of (A) forest shade (n = 24), (B) small gaps (n = 15), and (C) cloudy (n = 3) light environments collected at primary display perches of long–tailed manakins. Vertical bars indicate standard errors.

Figure 6. Long–distance conspicuousness as measured by chromatic contrast against the background (A) and achromatic contrast against the background (B) for different body regions of male (solid bars; n = 62) and female (open bars; n = 59) long–tailed manakins in a forest shade light environment and against a background of green leaves. Standard errors are shown.

Figure 7. Short–distance conspicuousness as measured by chromatic contrast between body regions (A) and achromatic contrast between body regions (B) for male (solid bars; n = 62) and female (open bars; n = 59) long–tailed manakins in three different light environments. Standard errors are shown.

Figure 8. Mean chromatic contrast against the background for male (A; n = 62) and female (B; n = 59) long–tailed manakins for a background composed of green leaves (shaded bars) or brownish bark (diagonally striped bars) in three different light environments. Standard errors are shown.

Figure 9. Mean achromatic contrast against the background for male (A; n = 62) and female (B; n = 59) long–tailed manakins for a background composed of green leaves (shaded bars) or brownish bark (diagonally striped bars) in three different light environments. Standard errors are shown.

Figure 10. Component loadings for the first three principal components (PC1, PC2, and PC3) of a principal components analysis (PCA) of irradiance spectra from three different light environments (Fig. 3) measured at primary display perches of long–tailed manakins.

Figure 11. Irradiance PC scores from spectra collected in forest shade (A) and small gap (B) light environments at primary (solid bars), secondary (open bars), control (diagonally striped bars), and arbitrary (shaded bars) display perches of long–tailed manakins (see text for explanation of perch types). Standard errors are shown.

Figure 12. Comparison of physical characteristics of primary, secondary, and control perches of long–tailed manakins (see text for explanation of perch types).

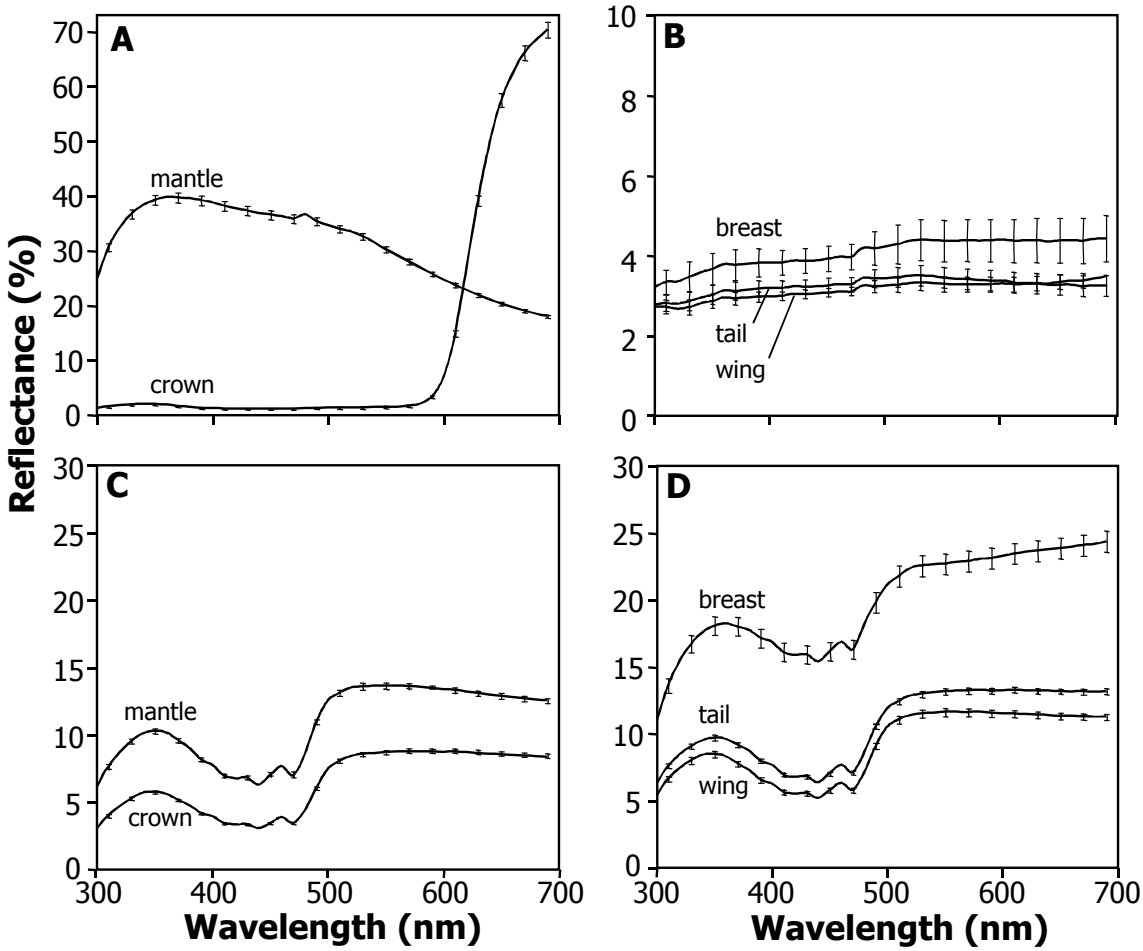
A
mantle
crown
B
breast
tail
wing
C
mantle
crown
D
breast
tail
wing
Reflectance (%)
Wavelength (nm)
Wavelength (nm)

Figure 1

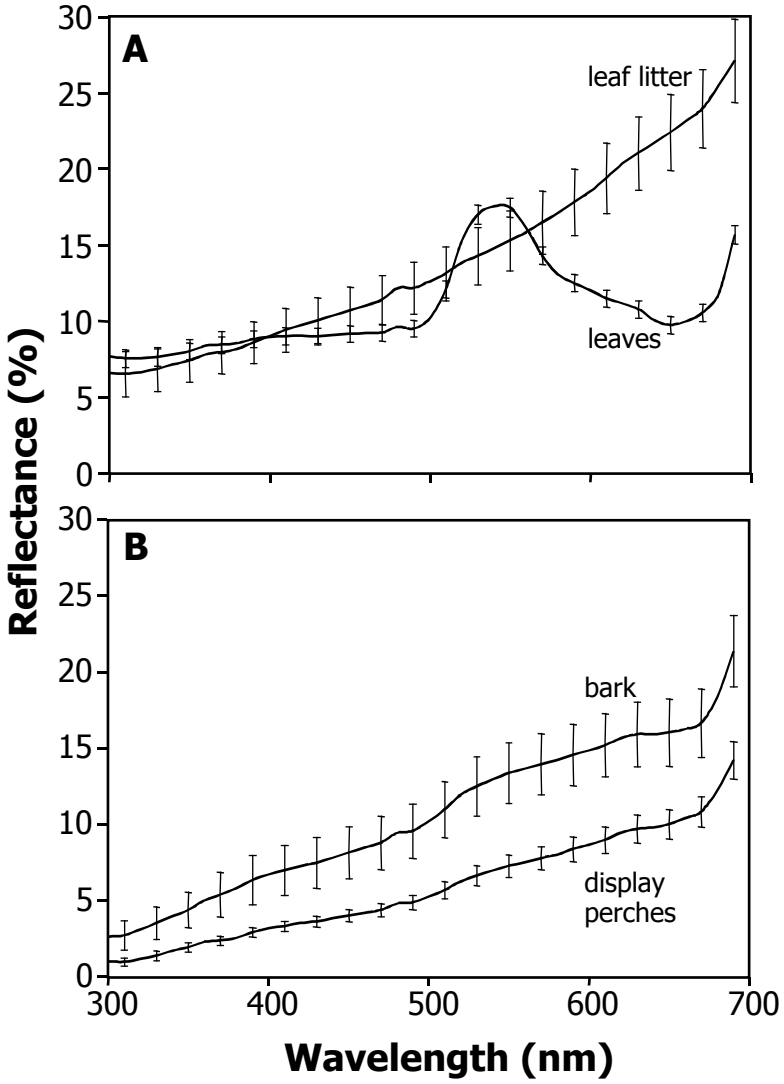
A
leaf litter
leaves
B
bark
display
perches
Reflectance (%)
Wavelength (nm)
0
5
10
15
20
25
30
300
400
500
600
700

Figure 2

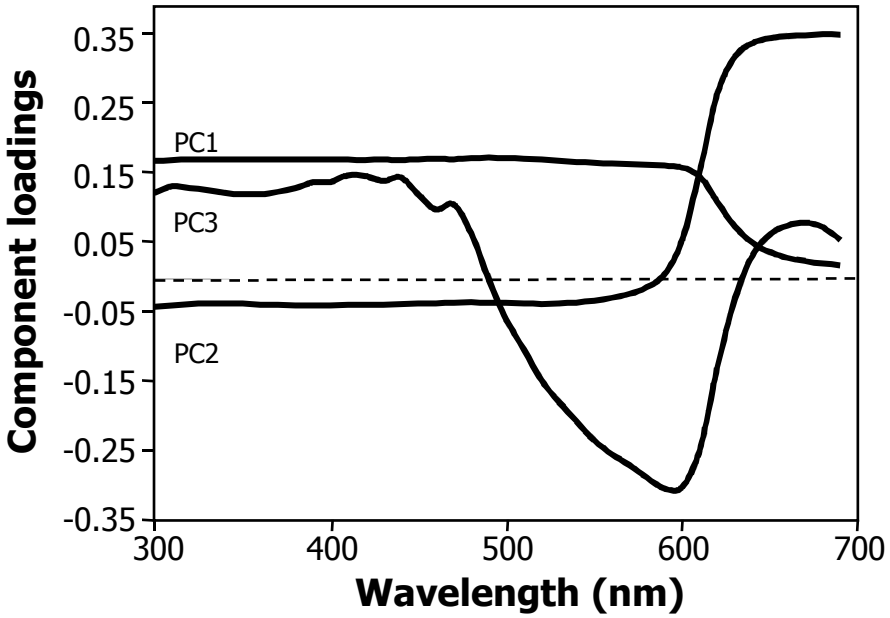
0.35
0.25
0.15
0.05
-0.05
-0.15
-0.25
-0.35
Component loadings
PC1
PC3
PC2
300
400
500
600
700
Wavelength (nm)

Figure 3

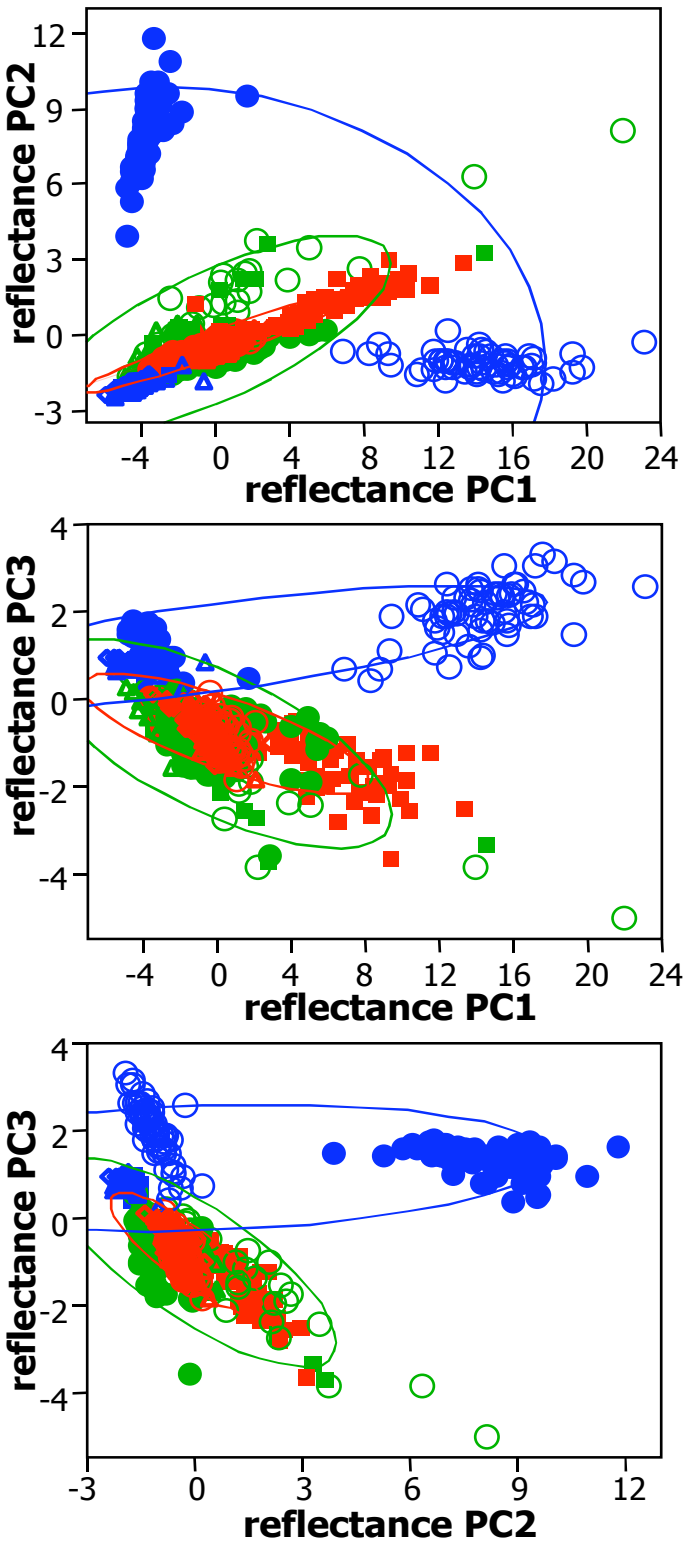
reflectance PC2
reflectance PC1
reflectance PC3
reflectance PC1
reflectance PC3
reflectance PC2

Figure 4

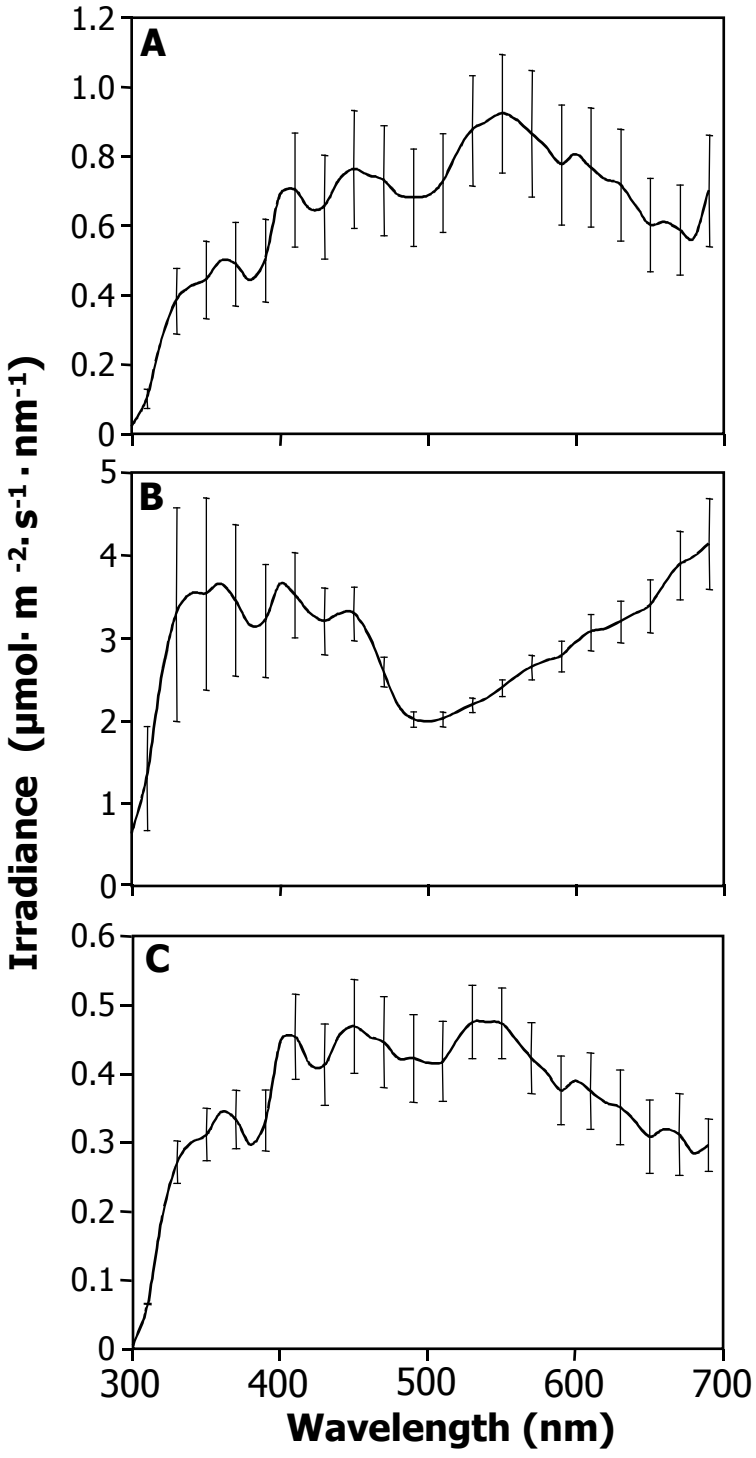
A
B
C
Irradiance (µmol· m -2· s-1 · nm-1)
Wavelength (nm)
1.2
1.0
0.8
0.6
0.4
0.2
0
5
4
3
2
1
0
0.6
0.5
0.4
0.3
0.2
0.1
0
300
400
500
600
700

Figure 5

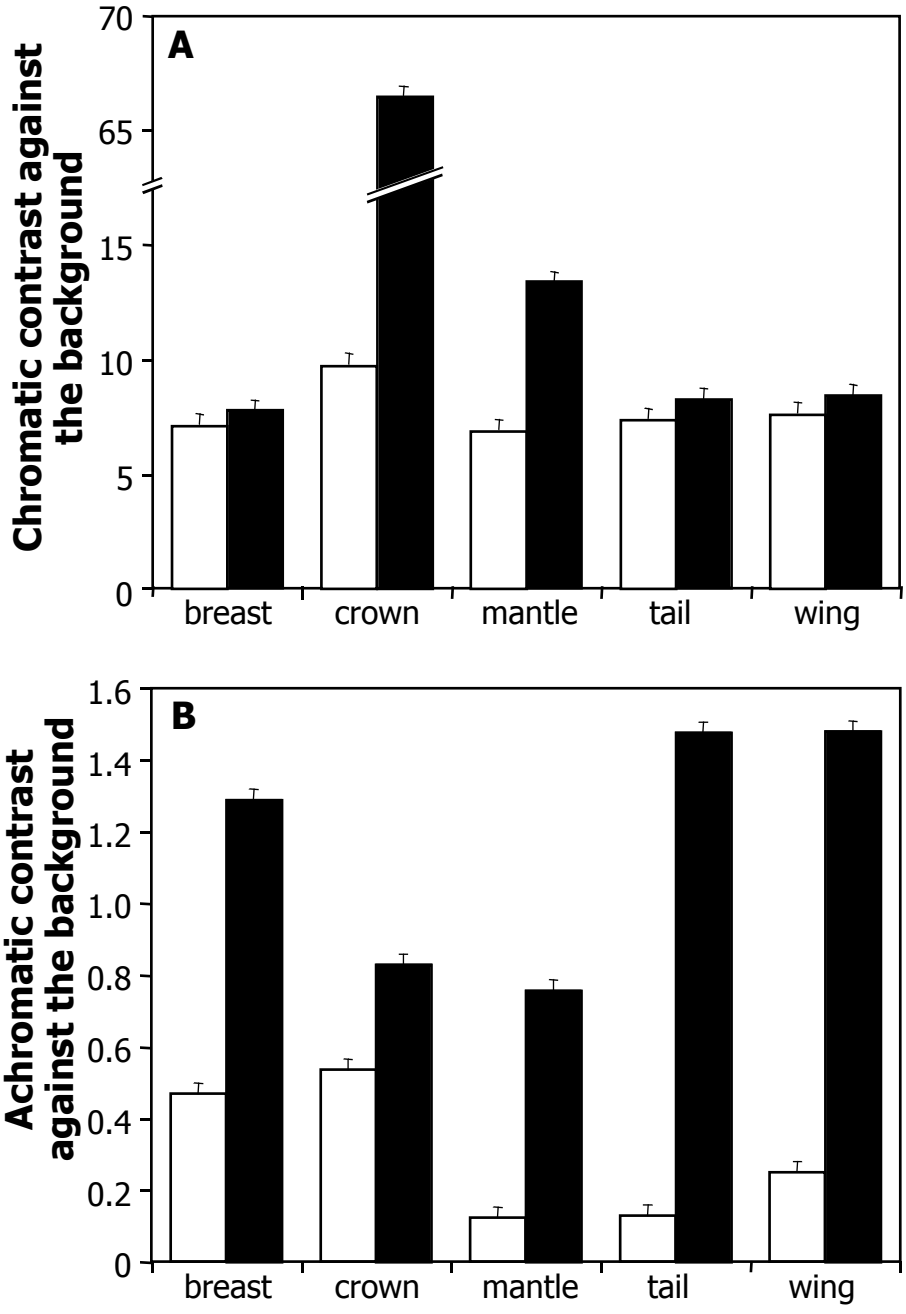
A
Chromatic contrast against the background
70
65
15
10
5
0
breast
crown
mantle
tail
wing
B
Achromatic contrast against the background
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
breast
crown
mantle
tail
wing

Figure 6

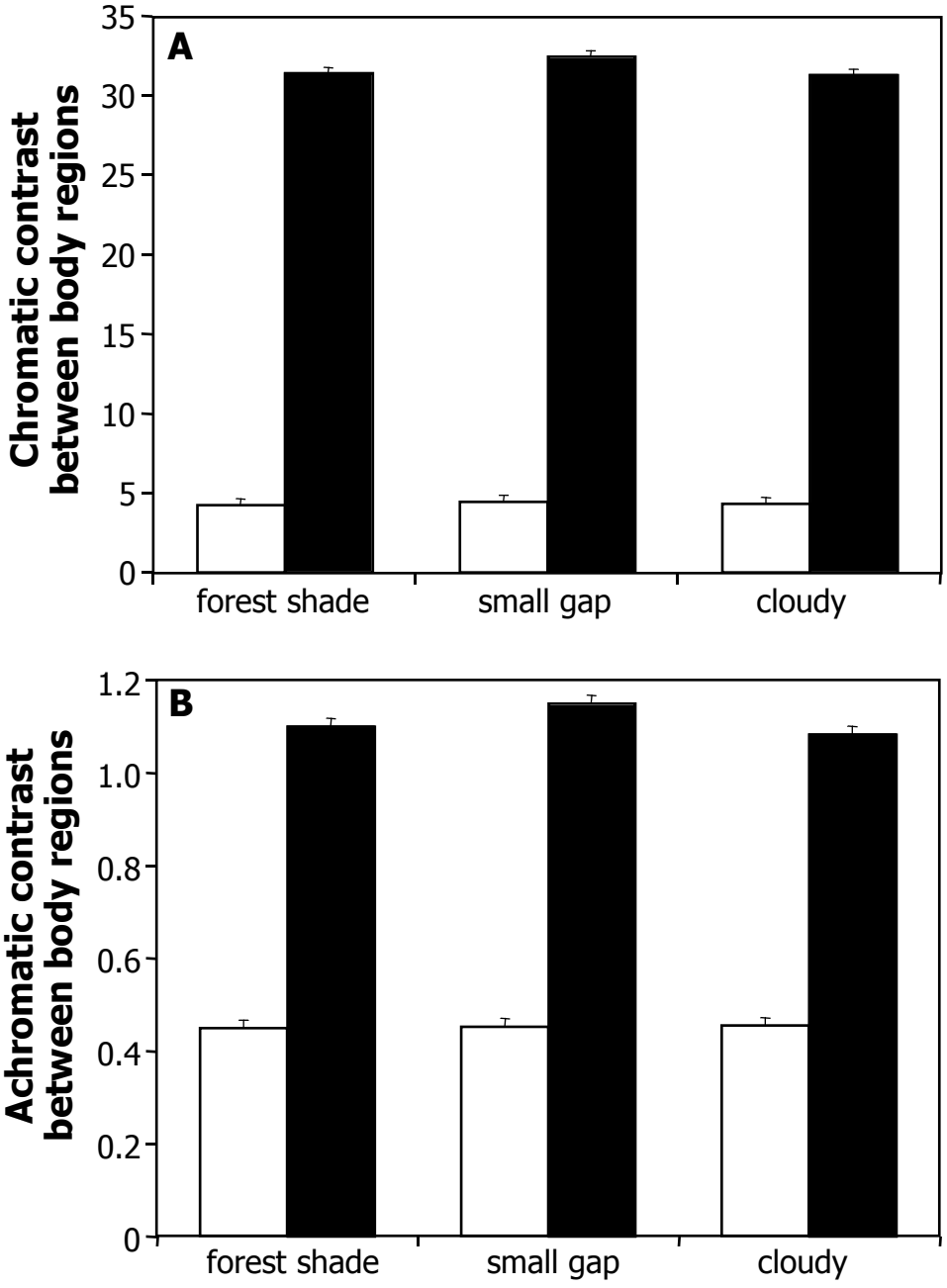
A
Chromatic contrast between body regions
35
30
25
20
15
10
5
0
forest shade
small gap
cloudy
B
Achromatic contrast between body regions
1.2
1.0
0.8
0.6
0.4
0.2
0
forest shade
small gap
cloudy

Figure 7

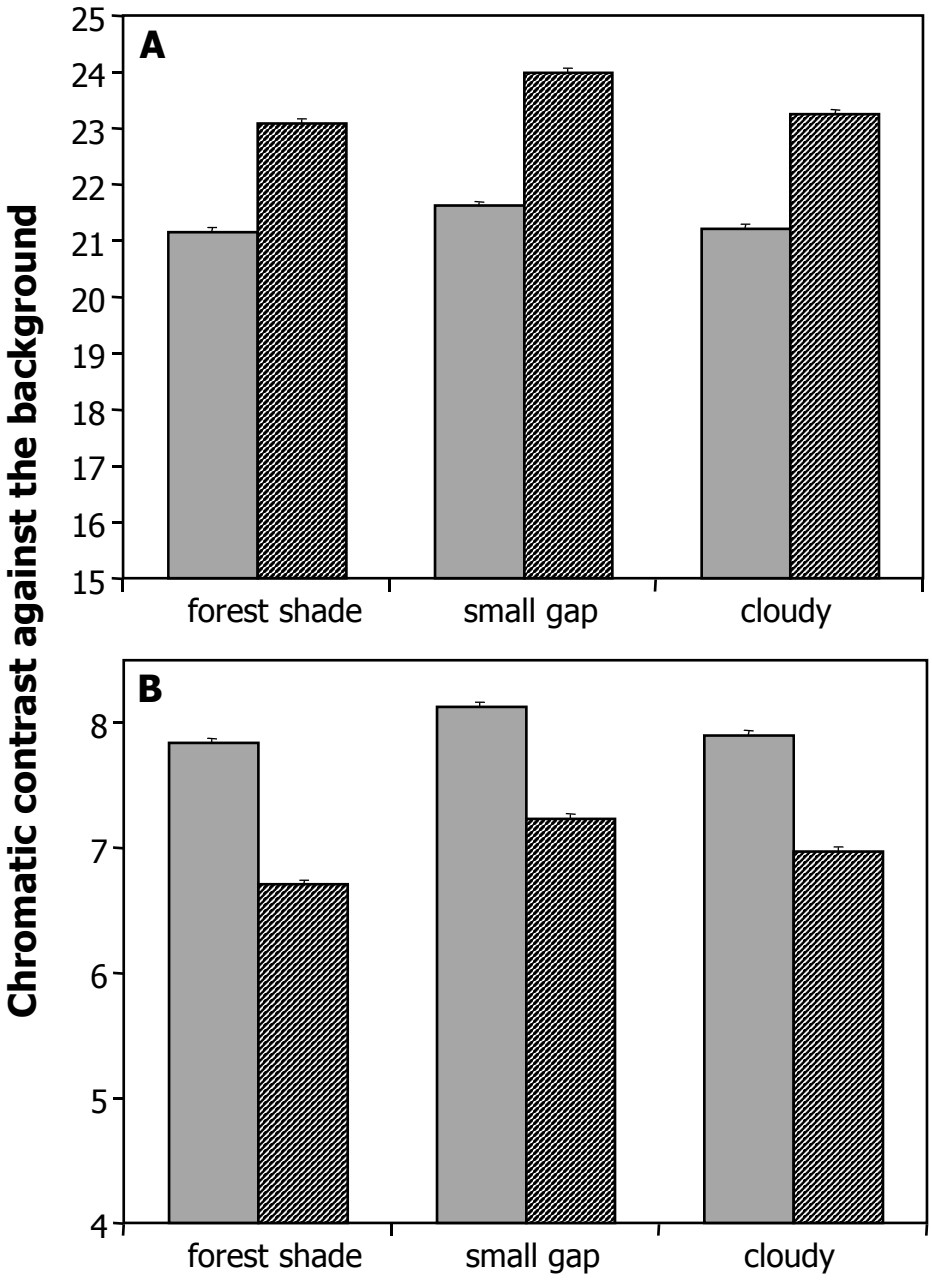
A
B
Chromatic contrast against the background
25
24
23
22
21
20
19
18
17
16
15
8
7
6
5
4
forest shade
small gap
cloudy
forest shade
small gap
cloudy

Figure 8

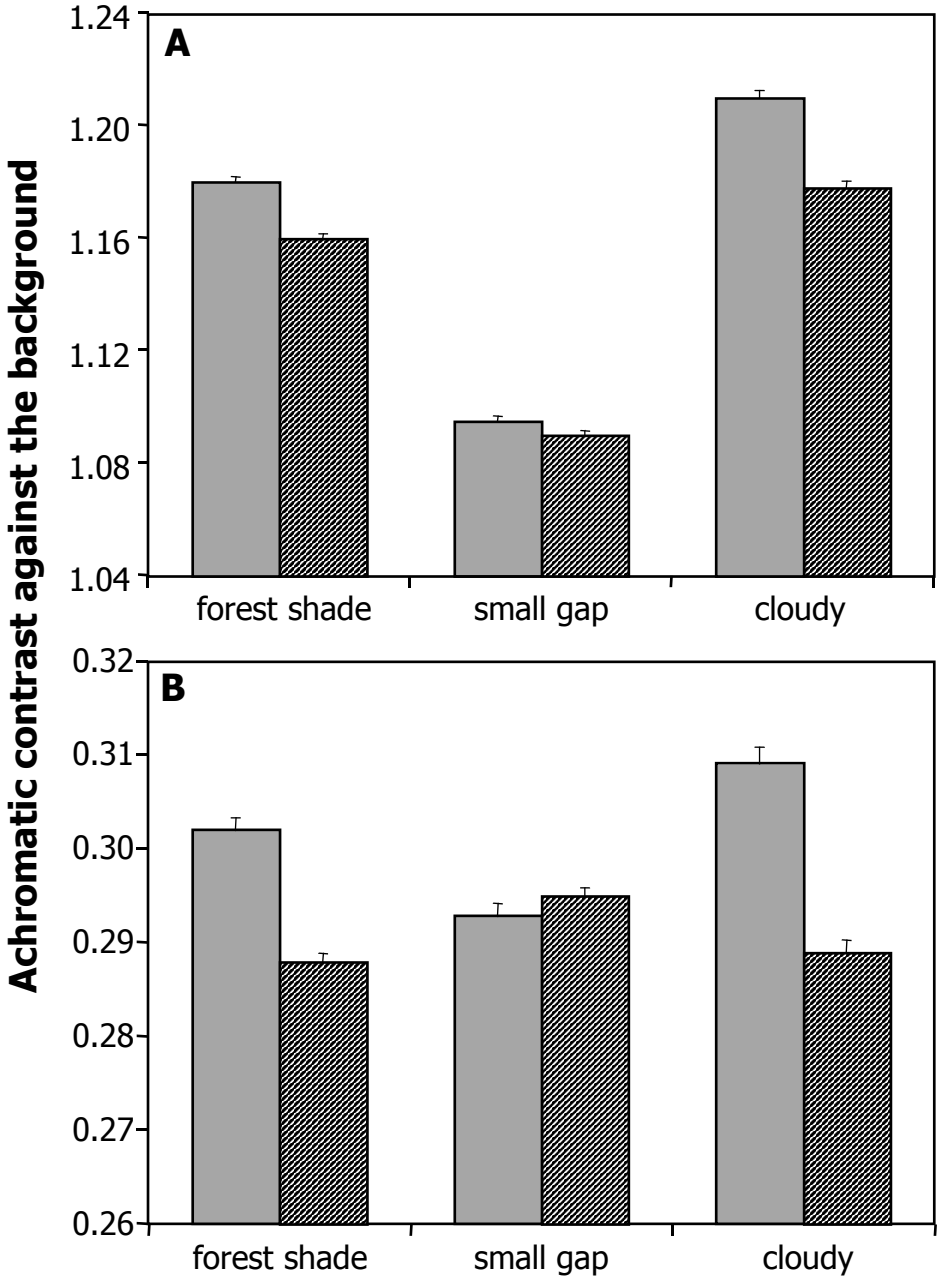
Achromatic contrast against the background
A
1.24
1.20
1.16
1.12
1.08
1.04
forest shade
small gap
cloudy
B
0.32
0.31
0.30
0.29
0.28
0.27
0.26
forest shade
small gap
cloudy

Figure 9

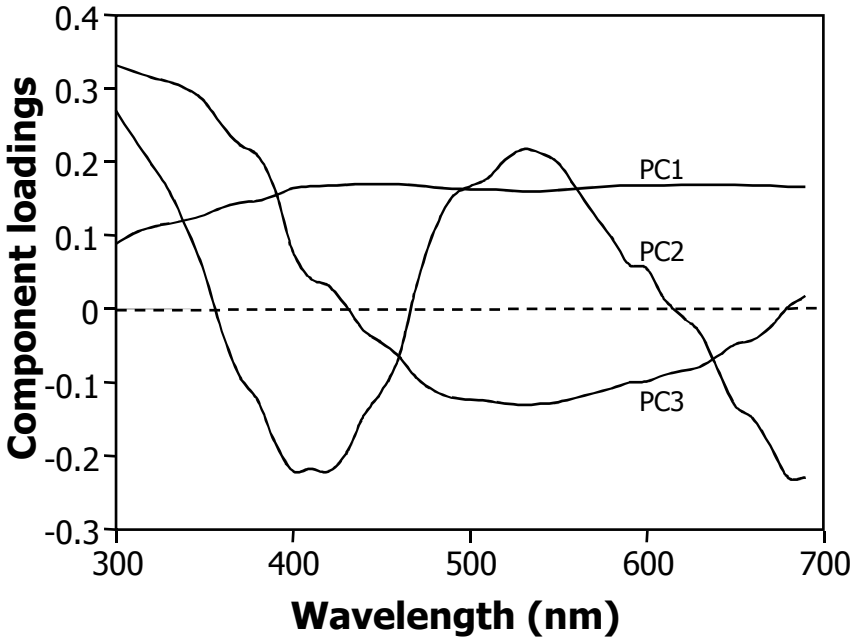
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
Component loadings
PC1
PC2
PC3
300
400
500
600
700
Wavelength (nm)

Figure 10

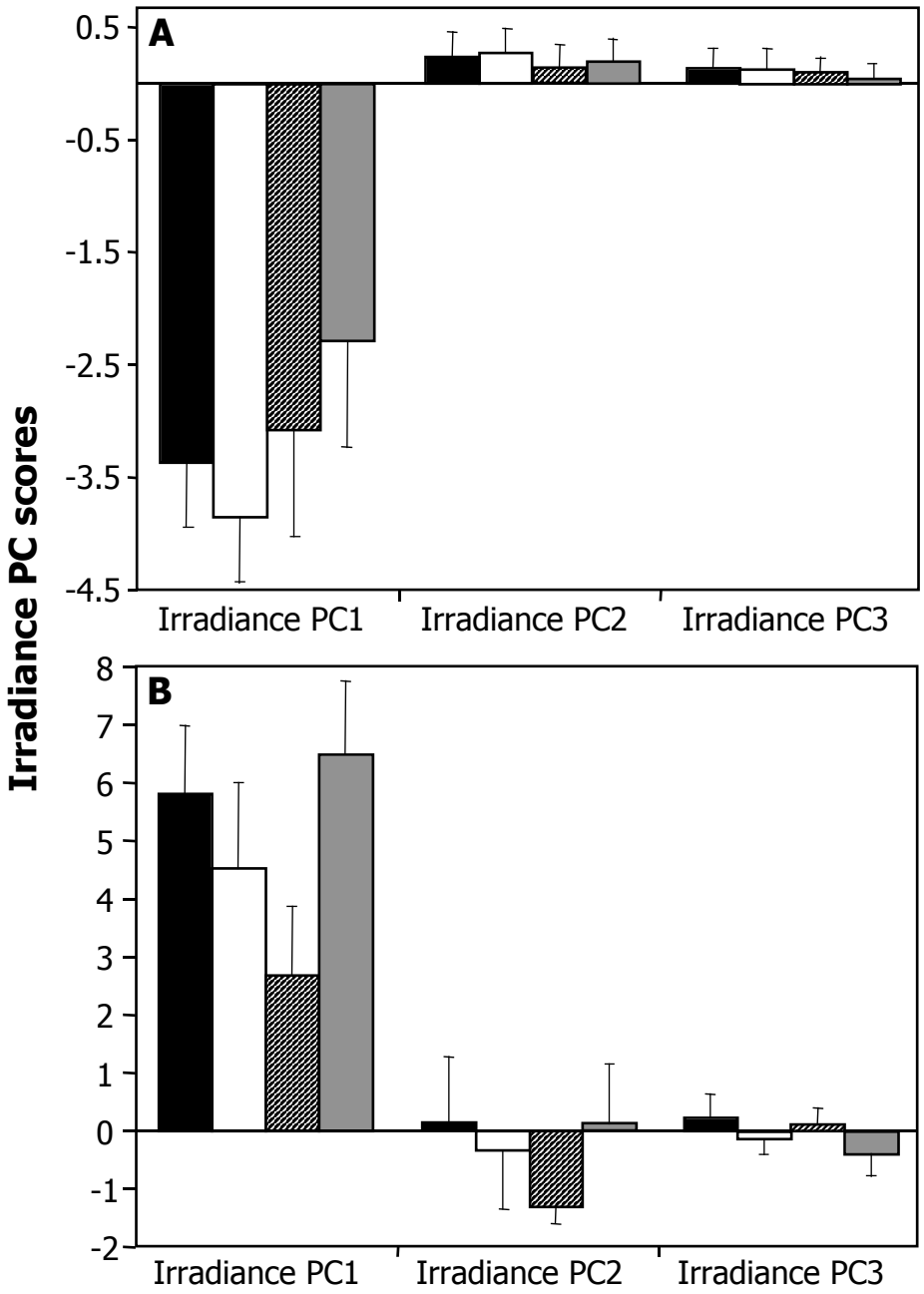
A
B
Irradiance PC scores
0.5
-0.5
-1.5
-2.5
-3.5
-4.5
8
7
6
5
4
3
2
1
0
-1
-2
Irradiance PC1
Irradiance PC2
Irradiance PC3
Irradiance PC1
Irradiance PC2
Irradiance PC3

Figure 11

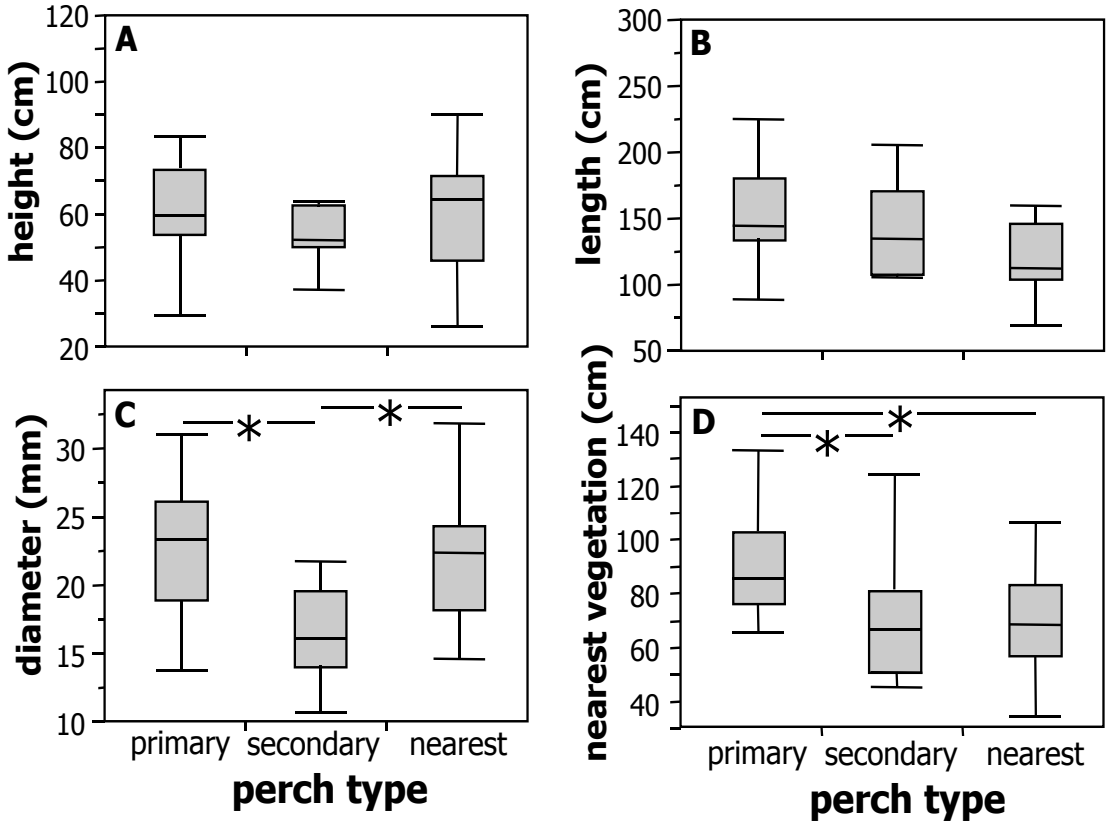
A
height (cm)
120
100
80
60
40
20
B
length (cm)
300
250
200
150
100
50
C
diameter (mm)
30
25
20
15
10
primary
secondary
nearest
perch type
D
nearest vegetation (cm)
140
120
100
80
60
40
primary
secondary
nearest
perch type

Figure 12

CHAPTER 4. CAROTENOID, MELANIN, AND STRUCTURAL PLUMAGE COLOR OF AVIAN STUDY SKINS: EFFECTS OF SPECIMEN AGE AND GEOGRAPHIC VARIATION

ABSTRACT

Museum specimens are an invaluable resource for taxonomic, systematic, and comparative studies and are increasingly being relied upon for novel research purposes. Researchers now routinely quantify the color of feathers on study skins to address a broad range of research questions, but few studies have investigated how well the plumage coloration of museum specimens reflects the coloration of wild birds. In this study, we used reflectance spectrometry to compare the reflectance of feathers on museum skins and the feathers of wild birds and to investigate the effects of specimen age on plumage coloration. We use the long–tailed manakin, Chiroxiphia linearis, as a model system for investigating these potential differences in color. Long–tailed manakins are ideal for this type of study because their colorful plumage patches result from the three primary plumage coloration mechanisms found in birds: melanin pigmentation, carotenoid pigmentation, and structural coloration. These features of long–tailed manakin plumage allowed us to independently assess variation in each plumage coloration mechanism. Reflectance spectra obtained from museum specimens were generally similar to those obtained from wild birds. Nonetheless, we found significant differences in coloration between museum skins and wild birds for all three mechanisms of plumage coloration. We identified three key sources of variation: 1) overall differences between museum specimens and wild birds, 2) geographic variation in color, and 3) variation in color according to specimen age. Changes in color were not evenly distributed across the reflectance spectrum but rather varied according to which color production mechanism was involved. We discuss the potential proximate causes of these changes in color, many of which apply to both museum specimens and wild birds, and identify the types of

studies are likely to be most sensitive to these changes. Our study therefore also provides the first extensive review of potential sources of color wear and fading in avian museum skins and wild birds.

INTRODUCTION

Studies based on museum specimens represent a large portion of the foundation of our understanding of the diversity of life on earth (Winker 2004). Museum specimens continue to be an invaluable resource for taxonomic, systematic, and comparative studies and are increasingly being used for previously unanticipated purposes (Winker 2004). Many contemporary studies use data from museum specimens as windows into the past, for example, to document the spread of infectious disease (e.g., Yates et al. 2002), to investigate the effects of nuclear disasters (Ellegren et al. 1997) or habitat degradation (Lens et al. 2002) on populations, and to assess ecosystem–level changes in the environment (Schell 2000). With museum specimens being put to such diverse and important uses, it seems reasonable to ask whether specimens are an accurate representation of wild animals. Clearly, for certain stable features such as skeletal size and genetic or isotopic signature, the answer is "yes". However, some features, such as the coloration of bare parts, fur, or feathers of museum study skins, might be expected to change over time (Test 1940; Gabrielson and Lincoln 1951).

Evaluating variation in the color of feathers on avian museum skins forms the basis for a broad range of research questions. The color and pattern of plumage preserved on study skins can be used as a taxonomic marker (e.g., Brumfield and Remsen 1996; Prum 1997; Johnson et al. 1998; Brumfield and Braun 2001; Patten and Unitt 2002), to sex or age birds (e.g., Wood 1992; Jeffrey et al. 1993; Doucet et al. *In review*), or to document plumage reflectance patterns of a particular species or group of species (e.g., Brumfield et al. 2001). Moreover, color itself is often the primary target of museum–based studies. Researchers have measured the color of feathers on study skins,

for example, to reveal unusual reflectance patterns (Finger and Burkhardt 1994; McGraw 2004), to document historical changes in color (Hudon and Brush 1989), to examine plumage reflectance and sexual dichromatism from the visual perspective of an avian receiver (Mahler and Kempenaers 2002; Eaton and Lanyon 2003), or to test broad–scale predictions about the evolution of particular types of color signals (Endler and Théry 1996; Owens and Hartley 1998; Hausmann et al. 2003; Heindl and Winkler 2003; Gomez and Théry 2004). These studies highlight the importance of ascertaining that the plumage colors of study skins are representative of the coloration of wild birds.

Until recently, studies of plumage coloration involving study skins or wild birds relied on human visual assessments of color. It has become increasingly clear, however, that the avian visual system differs substantially from that of humans (Cuthill et al. 2000; Hart 2001; Cuthill 2006). Most notably, in addition to cone photoreceptors analogous to the red, blue, and green cone photoreceptors of humans, birds have a fourth, short–wavelength sensitive cone photoreceptor that, combined with ocular media that allow the transmission of ultraviolet wavelengths, confers birds with the ability to see ultraviolet wavelengths (Cuthill et al. 2000; Hart 2001; Cuthill 2006). Moreover, cone photoreceptors in birds are associated with oil droplets that narrow their spectral sensitivities and thereby increase wavelength discrimination (Cuthill et al. 2000; Hart 2001; Cuthill 2006). Birds also have double cone photoreceptors that are thought to facilitate the discrimination of achromatic contrast (Cuthill et al. 2000; Hart 2001; Cuthill 2006). These fundamental differences between human and avian vision have led to the development of improved methods for quantifying plumage coloration, such as full–spectrum reflectance spectrometry (Bennett et al. 1994; Cuthill et al. 1999).

Our goal in this study was to determine how well the plumage coloration of museum skins represents the plumage coloration of wild birds. There have been a number of anecdotal observations of dulling, fading, and foxing of museum specimens in the literature (Gabrielson and Lincoln 1951; Endler and Théry 1996; Mahler and Kempenaers 2002; Eaton and Lanyon 2003; Hausmann et al. 2003; Jouventin et al. 2005). Until recently, however, there had been no systematic attempt to compare the coloration of feathers on museum skins and on wild birds (McNett and Marchetti 2005). Moreover, potential sources of variation in plumage color between study skins and wild birds have never been comprehensively reviewed. Here, we use full–spectrum reflectance spectrometry to compare the plumage reflectance of wild–caught long–tailed manakins *Chiroxiphia linearis*, with museum skins of this species.

Long–tailed manakins are ideal for this type of investigation, as their colorful plumage patches are produced by the three primary coloration mechanisms found in birds: carotenoid pigmentation, melanin pigmentation, and structural coloration. Definitive–plumaged adult males have bright red carotenoid–based crown patches, structural sky–blue mantles, and melanin–based black body plumage and flight feathers (Doucet et al. 2006). These features of manakin plumage ornamentation allowed us to independently assess the potential effects of wear or fading of plumage colors resulting from each of these mechanisms. Because our sample of study skins included individuals from throughout the range of long–tailed manakins, we also assessed the potentially confounding effect of geographic variation in ornamentation.

METHODS

Study system

Long–tailed manakins are distributed along the Pacific coast of North and Central America from southeastern Mexico to northwestern Costa Rica (Fig. 1). The sexes are strongly sexually dichromatic and adult males are adorned with a remarkable array of elaborate sexual ornaments. While female plumage is dull olive–green, adult males are ornamented with a scarlet crown patch, a sky–blue mantle, velvety black body plumage, bright orange legs, and elongated central tail plumes. Males progress through a series of age–specific predefinitive plumages before attaining their definitive adult plumage in their fifth year (Doucet et al. *In review*).

We used biochemical and microscopic techniques to confirm the mechanisms of color production in feathers collected from male long–tailed manakins in definitive adult plumage. Most red plumage in passerines birds results from the deposition of carotenoids into growing feathers (McGraw 2006a). To confirm that this mechanism was also responsible for the red crown coloration of adult male long–tailed manakins, we used a thermochemical extraction procedure and high performance liquid chromatography to identify specific carotenoids in the red crowns of adult males (S. M. Doucet and K. J. McGraw, unpubl. data, McGraw et al. 2004a; McGraw et al. 2005). Most black plumage in birds results from the deposition of melanin pigments into growing feathers (McGraw 2006b). To confirm that this mechanism was also responsible for the black coloration of body and flight feathers in adult male long–tailed manakins, we compared the reflectance spectra of black body regions in long–tailed manakins to known melanin–based spectra (Mennill et al. 2003; McGraw 2006b) and confirmed the presence of pigment granules of

the appropriate shape, size, and density in transmission electron micrographs (S. M. Doucet, unpubl. data, Doucet et al. 2004). All non–iridescent blue plumage colors in birds investigated to date result from the constructive interference of light scattered by materials of different refractive indices (air and keratin) within the medullary keratin matrix of feather barbs (Prum 2006). To confirm that this mechanism was also responsible for blue coloration in long–tailed manakins, we immersed blue feathers in liquid of the same refractive index as keratin and found that the blue color disappeared, demonstrating that the color is produced structurally (Mason 1923; Shawkey and Hill 2005). We also used transmission electron microscopy to identify the presence of a medullary keratin matrix similar to that known to produce blue and ultraviolet coloration in several passerine birds (Prum et al. 1999; Prum et al. 2003; Shawkey et al. 2003a; Doucet et al. 2004).

Field methods

From March to July 2003 and 2004, we studied long–tailed manakins at Santa Rosa National Park, Guanacaste Conservation Area, Costa Rica (10˚ 40' N, 85˚ 30 W). We used mist nets to capture 293 manakins at or near lek sites (all birds captured between 0500 and 1200 CST). We fitted each bird with a numbered aluminum leg band and a unique combination of three colored leg bands. We measured the spectral reflectance of wild birds using an Ocean Optics USB 2000 spectrometer and PX–2 flash lamp (Ocean Optics, Dunedin, FL). Our reflectance probe was mounted in a black rubber probe holder, which excluded all external light and maintained the probe at a fixed distance (5 mm) from, and perpendicular to, the feather surface. We measured the reflectance of five body

regions, namely the crown, mantle, tail, wing, and breast of each bird. We recorded five measurements per body region, each of which comprised an average of 10 readings collected in rapid succession by the operating software. Because the breast, wing, and tail were of similar coloration within birds, we averaged these together and refer to them as 'body color' throughout. All reflectance data are expressed as the percentage of reflectance from a white standard (WS–1, Ocean Optics). We collected 5–10 crown and mantle feathers and the right outer rectrix of each bird for biochemical and structural analysis of color production mechanisms (described above).

Museum

We visited the collections of the Louisiana State University Museum of Natural Science in March of 2003 and the American Museum of Natural History in January of 2004. We measured the plumage reflectance of the study skins of 122 long–tailed manakins as described above. We also recorded the date and location of collection indicated on specimen tags when available. We then obtained the latitude and longitude coordinates of all specimen collection locations and recorded these to the nearest degree. If only a broad region such as a state or province was identified on specimen tags, we recorded the coordinates of the approximate center of the distribution of long–tailed manakins within that region.

Colorimetric variables

For each body region, we calculated three color variables to approximate the three principal dimensions of color: brightness, hue, and saturation (Hailman 1977; Andersson

and Prager 2006; Montgomerie 2006a). For all body regions, we calculated brightness as the total reflectance across the bird visible spectrum from 300 nm to 700 nm. We calculated saturation, a measure of spectral purity, as the maximum reflectance minus the minimum reflectance divided by the total reflectance. Because of the large differences in the shape of the reflectance spectra between different body regions (Fig. 2), we used different means of calculating hue for each region in order to ensure that this variable captured relevant information. For the blue mantle and black body, we calculated hue as the wavelength of maximum reflectance. For the red crown, we calculated hue as the wavelength at which reflectance reached 50 % of its maximum reflectance. To examine whether variation in reflectance between wild birds and study skins was evenly distributed across the visual spectrum of birds, we calculated four measures of chroma (e.g., Hill et al. 2005). To do this, we divided the visual spectrum into four 100–nm segments and divided the reflectance in each segment by the total reflectance. We refer to these as UV–chroma (300–400 nm), blue chroma (400–500 nm), green chroma (500–600 nm), and red chroma (600–700 nm).

Statistical Analysis

Only males in definitive adult plumage had a fully–developed red crown, an entirely blue mantle, and entirely black body plumage (Doucet et al. *In review*). We therefore restricted our analyses to these males. Because the distribution of long–tailed manakins follows a roughly northwest to southeast axis (Fig. 1), latitude and longitude coordinates of sampling locations were highly correlated ($r_s = 0.87$, $n = 111$, $P < 0.0001$). We used principal components analysis to combine these two variables into one index of

location. Both latitude and longitude loaded highly and positively (component loadings = 0.71) on the first principal component (PC1), which explained 98% of the variation in these coordinates. We therefore used PC1 as a measure of geographic location in our analyses. High PC1 location scores correspond to individuals sampled from the northwestern portion of the range of long–tailed manakins, while low PC1 location scores correspond to individuals sampled from the southeast portion of the range (Fig. 1). We used Shapiro–Wilk tests to determine whether the distribution of the original variables, or of their residuals, met normality assumptions as required by t–tests or regression analyses, respectively. In many cases where variable distributions departed significantly from normality, standard transformations did not improve the fit to normality. We therefore used non–parametric tests where appropriate. Slight variations in sample size arose because not all information was available from all individuals/specimens.

RESULTS

Plumage reflectance of long–tailed manakins

Adult male long–tailed manakins have highly saturated red crown patches that reflect very little at short and medium wavelengths, but increase steeply in reflectance at long wavelengths (Fig. 2). By contrast, their blue mantles reflect maximally at ultraviolet wavelengths and exhibit lower reflectance at long wavelengths (Fig. 2). The black body plumage of adult male manakins exhibits low, uniform reflectance across the bird–visible spectrum (Fig. 2).

Overall differences between museum specimens and wild birds

There were significant differences in plumage coloration between museum skins and wild male long–tailed manakins in definitive plumage. In particular, wild males had significantly brighter and more highly saturated red crown coloration than study skins (Table 1, Figs. 2, 3). Wild males also had significantly brighter blue mantles that peaked at shorter (more UV) wavelengths than study skins (Table 1, Figs. 2, 3). The black body coloration of live–caught males was also significantly darker and less saturated and peaked at significantly shorter wavelengths than that of museum skins (Table 1, Figs. 2, 3).

Effects of specimen age and geographic location

To determine whether differences in plumage coloration between wild males and study skins might have resulted from degradation in color over time, we investigated the relationship between plumage color variables and the age of the specimens. Only the hue of the black body plumage was significantly correlated with the age of the specimens, with the hue of older specimens peaking at longer wavelengths than that of younger specimens (Table 2).

To determine whether plumage color varied geographically among long–tailed manakins, we investigated the relationship between plumage color variables and sampling location (location PC1 – see methods for PCA details). The brightness and saturation of the crown were significantly negatively correlated with location PC1, such that males in the southeastern portion of the range of long–tailed manakins (Costa Rica) had brighter and more highly saturated red crowns than males in the northwestern portion

of the range (Mexico; Table 3). Additionally, the brightness of the mantle was significantly negatively correlated with location PC1, while hue was positively correlated with PC1, such that males in the southeastern portion of the range had brighter blue mantles that peaked at shorter (more UV) wavelengths than males in the northwestern portion of the range (Table 3). None of the black body plumage variables were significantly correlated with geographic location (Table 3).

Because plumage color varied geographically (see above) and specimen age was significantly related to sampling location ($r_s = 0.56$, $n = 104$, $P < 0.0001$), we used multiple regression analyses to investigate the relative influence of specimen age and geographic variation on plumage coloration. Variance inflation factors were less than 1.15 in all of our multiple regression analyses, indicating that colinearity did not unduly bias our analyses. When controlling for geographic variation in color, there was a significant negative relationship between crown saturation and specimen age, such that older specimens had significantly less saturated red crowns than younger specimens (Table 4, Fig. 4). Additionally, there was a significant negative relationship between mantle brightness and specimen age whereas there was a significant positive relationship between mantle hue and specimen age, such that older specimens had duller blue mantle plumage reflectance that peaked at longer wavelengths (Table 4, Fig. 4). There were also significant positive relationships between the brightness, hue, and saturation of the black body plumage and specimen age such that older study skins had lighter, less uniform body plumage reflectance that peaked at longer wavelengths (Table 4, Fig. 4). Similar relationships between plumage coloration and specimen age were obtained when only birds from the southern end of the distribution are considered (unpubl. data).

These multiple regression models also allowed us to reassess the relationship between sampling location and variation in color. When controlling for the effects of specimen age, there was a significant negative relationship between crown brightness and sampling location, such that birds in the southeastern portion of the range had brighter red crowns than birds in the northwestern portion of the range (Table 4, Fig. 5). Similarly, there was a significant negative relationship between mantle brightness and sampling location, such that birds in the southeastern portion of the range had brighter blue mantles than birds in the northwestern portion of the range (Table 4, Fig. 5). There was no significant influence of sampling location on the coloration of the black body plumage (Table 4, Fig. 5).

To determine whether the relative influence of specimen age and geographic location on plumage color varied across the bird–visible spectrum, we used multiple regression models to investigate the relationship between four measures of chroma and both specimen age and sampling location for each body region. When controlling for geographic variation in color, there was a significant positive relationship between UV–, blue–, and green–chroma of the red crown and specimen age (Table 5). By contrast, there was a significant negative relationship between red–chroma of the red crown and specimen age (Table 5). Thus, the red crown of older specimens reflected proportionally less in the red portion of the spectrum and proportionally more in the green, blue, and UV regions of the spectrum. Additionally, there was a significant negative relationship between UV–chroma of the blue mantle and specimen age (Table 5). By contrast, there was a significant positive relationship between green– and red–chroma of the blue mantle and residual specimen age (Table 5). Thus, the blue mantle of older specimens reflected

proportionally less in the UV and proportionally more in the green and red regions of the spectrum. There was also a significant negative relationship between blue–chroma of the black body plumage and specimen age. By contrast, there was a significant positive relationship between red–chroma of the black body plumage and specimen age. Thus, the black body plumage of older specimens reflected proportionally less in blue and green portions of the spectrum and proportionally more in red regions of the spectrum.

When controlling for the effects of specimen age, there was a significant positive relationship between UV–chroma of the red crown and location PC1, such that the crowns of birds in the northwestern portion of the range reflected proportionally more in the UV than those of birds in the southeastern portion of the range (Table 5). There was no significant relationship between any of the chroma variables for the blue mantle and location PC1. However, there was a significant positive relationship between UV–chroma of the black body plumage and location PC1 whereas there was a significant negative relationship between blue– and green–chroma of the black body plumage and location PC1. Thus, the black body plumage of birds in the northwestern part of the range reflected proportionally more in the UV and proportionally less in the blue and green regions of the spectrum than birds in the southeastern portion of the range.

DISCUSSION

Using long–tailed manakins as a model system, we show that there are significant differences in plumage color between museum specimens and wild birds with respect to carotenoid, melanin and structural plumage coloration. Our data suggest that these differences are due in part to a combination of geographic variation and specimen age.

Colors produced by different mechanisms also showed spectrally different patterns of color degradation.

Some of the differences in coloration between the feathers of wild males and feathers on study skins may result from the specimen preparation process *per se*. It is possible, for example, that the feathers of study skins have been stripped of their preen gland secretions. These uropygial oils create a protective sheen on the feathers (Elder 1954) that could potentially affect plumage brightness (Piersma et al. 1999; Reneerkens and Korsten 2004; Montgomerie 2006b). Moreover, because some uropygial oils do not absorb light evenly across the reflectance spectrum, their presence or lack thereof on feathers has the potential to influence the shape of reflectance spectra (Piersma et al. 1999; Reneerkens and Korsten 2004; Montgomerie 2006b). The preen oil secretions could be destroyed during the specimen preparation process through absorption by the corn meal or potato flour used to prepare specimens or by chemical breakdown when detergents are used to clean the skins. Alternatively, preen oils might simply degrade over time. Similarly, several taxonomic groups (mostly non–passerines) have powder down patches that secrete a greasy powdery substance thought to have similar functions to uropygial secretions (Wetmore 1920; Lowery and O'Neill 1966; Brown and Toft 1999). It is therefore possible that secretions from powder down, or lack thereof, would influence the plumage coloration of study skins in some species. The potential effects of uropygial secretions and powder down secretions on plumage reflectance need to be investigated in more detail. It should be noted that washing museum skins during the specimen preparation process is often essential to remove blood, fat, or dirt from feathers, and that, in most cases, any plumage color differences caused by the lack of preen or

powder down secretions are likely to be smaller than differences caused by heavily soiled plumage (Lowery and O'Neill 1966; Reneerkens and Korsten 2004; Montgomerie 2006b).

Differences between prebasic (postbreeding) and prealternate (prebreeding) plumages aside, overall color differences between study skins and wild birds could also result from the different times of year at which samples were collected. In this study, all wild birds were measured within a four–month period during the early part of the breeding season. By contrast, study skins were collected throughout the year, and might be expected to show more variation in feather wear, a factor known to influence plumage color (Örnborg et al. 2002; McGraw and Hill 2004). Within–year variation in color caused by wear is expected to be even greater in species that attain a bright nuptial plumage by wearing down the melanized tips of their feathers (e.g., Lyon and Montgomerie 1995; Willoughby et al. 2002). These effects underscore the importance of considering annual variation in plumage color when measuring museum specimens and could be minimized by comparing only samples collected at similar times of year.

Although the specimen preparation process has the potential to influence the color of study skins, our findings suggest that this is not the only reason for differences in feather color between study skins and wild birds. In analyses controlling for the age of the specimens, we found significant geographic variation in color for the red crown, blue mantle, and black body plumage. In particular, the brightness of the red crown increased when moving away from the northwestern portion of the range long–tailed manakins (Mexico) and toward the southeastern portion of the range (Costa Rica). Similarly, the brightness of the blue mantle increased toward the southeastern portion of the range.

Finally, the UV–chroma of the black body plumage increased, whereas the blue– and green–chroma decreased, toward the southeastern portion of the range. This geographic variation in color generally corresponds to subspecific distinctions in this species: *C. l. fastuosa*, which occupies the southeastern part of the range from El Salvador to Costa Rica, is described as being "brighter" that the nominate race, *C. l. linearis*, which occupies the northwestern part of the range from Guatemala to Mexico (Snow 2004). Our data suggest, however, that plumage color changes gradually across the range of long–tailed manakins rather than showing discontinuous variation between the two subspecies (see also Zink 2004). Elucidating the potential causes of this geographic variation is beyond the scope of the present study. Nonetheless, geographic variation in long–tailed manakin plumage coloration is subtle compared to that documented in other species, and this potentially confounding variable should be considered in future studies (e.g., Peterson 1997).

When controlling for geographic variation in color, we found significant relationships between various aspects of plumage color and the age of study skins. Older long–tailed manakin museum specimens had less saturated red crowns, blue mantles that peaked at longer (less UV) wavelengths and black body plumage that was more saturated (less spectrally flat), brighter (less dark), and peaked at longer wavelengths. Older museum specimens also had red crowns that reflected proportionally less in red regions of the spectrum and proportionally more in UV, blue, and green regions of the spectrum, blue mantles that reflected proportionally less in the UV and proportionally more in green and red regions of the spectrum, and black body plumage that reflected proportionally less in blue regions of the spectrum and proportionally more in red regions of the

spectrum. These documented relationships between plumage coloration and specimen age in long–tailed manakins suggest that the plumage colors fade or degrade over time. The most likely causes of such time–dependent patterns of color degradation are the accumulation of dust or dirt on the specimens (Eaton and Lanyon 2003; Montgomerie 2006a) or the abrasion or breakage of feather barbs or barbules (Örnborg et al. 2002; McGraw and Hill 2004). A few other possibilities exist. First, plumage coloration might be altered by the actions of feather–degrading bacteria (Shawkey and Hill 2004; Shawkey et al. *In review*). Uropygial oil has been shown to suppress the growth of feather–degrading bacteria and fungus (Pugh and Evans 1970; Pugh 1972; Shawkey et al. 2003b). Thus, in addition to the absence of maintenance behaviors like preening (Zampiga et al. 2004), the removal or degradation of uropygial secretions on museum skins effectively eliminates this protective mechanism. No study has specifically investigated the direct effects of feather chewing lice or mites on plumage coloration. However, correlational and experimental studies suggest an association between louse or mite abundance and plumage color (Thompson et al. 1997; Harper 1999; Figuerola et al. 2003). Although the effects of these ectoparasites on color could be indirect, it is also possible they could affect plumage coloration directly in a manner similar to feather–degrading bacteria. Bactericides and insecticides are sometimes used in specimen preservation and likely reduce the potential effects of keratinolytic bacteria, fungi, and feather–chewing ectoparasites.

Interestingly, the time–dependent effects of feather degradation influenced the three types of plumage color in different ways. Although the brightness of red and blue feathers did not significantly change with specimen age, the brightness of black feathers

increased with specimen age. Only the saturation of red and black feathers was significantly related to specimen age, whereas only the hue of blue and black feathers changed significantly with specimen age. Moreover, the proportional changes in color in different parts of the spectrum in relation to specimen age varied among the three different types of plumage color. Because feathers did not simply all become duller or change in color evenly across the spectrum, our findings suggest that the cause of color degradation varied, at least in part, according to the different color production mechanisms.

Carotenoid–based colors are produced by the deposition of carotenoid pigments into growing feathers (Fox and Vevers 1960; McGraw 2006a). The resulting color depends on the absorptive properties of the carotenoids in question (McGraw 2006a) and the underlying (usually white) structure of the feather (Shawkey and Hill 2005). Pigment concentration has been shown to affect the hue and saturation, but not the brightness, of carotenoid–based colors (Saks et al. 2003). The decrease in saturation and red chroma of the crown feathers of long–tailed manakins in older specimens, together with the increase in UV–, blue–, and green–chroma in these specimens, suggests that some degradation of the carotenoid pigments occurs over time. Carotenoids can degrade by oxidization or isomerization (Test 1940; Britton 1995). Oxidization breaks down carotenoid pigments and thereby results in pigment bleaching (Britton 1995). The significant decrease in the red–chroma and saturation of the red crown with increasing specimen age is consistent with such a bleaching effect. Not all carotenoids are equally reactive to oxidization (Britton 1995). The carotenoid responsible for the red crown color of long–tailed manakins (canthaxanthin; S. M. Doucet and K. J. McGraw, unpubl. data), for example,

oxidizes more slowly than one of the carotenoids responsible for epaulet color in red–winged blackbirds, *Agelaius pheniceus* (zeaxanthin, Britton 1995; McGraw et al. 2004a). Thus, pigment bleaching may be more pronounced in species where more reactive carotenoids are used as coloring pigments. Isomerization refers to changes in the stereochemistry (geometry) of carotenoid pigments (Britton 1995). Carotenoid pigments in nature, including those in bird feathers, are most often found in the all–*trans* form (Britton 1995; McGraw 2006a). Exposure to light, heat, oxygen, humidity, or acidity can promote the isomerization of carotenoids, resulting in less stable *cis*–isomers (Shi and Le Maguer 2000). The absorption maxima of carotenoid *cis*–isomers are often located at shorter wavelengths than those of trans isomers (Lyan et al. 2001; Updike and Schwartz 2003). Thus, *cis*–isomerization can produce changes in hue. Additionally, the instability of *cis*–isomers often results in their eventual degradation. Thus, some of our observed changes in carotenoid coloration may have occurred by pigment isomerization.

Non–iridescent structural colors are produced by the constructive interference of light in the medullary keratin matrix of feather barbs (Prum et al. 1999; Prum et al. 2003; Shawkey et al. 2003a; Doucet et al. 2004). In eastern bluebirds, *Sialia sialis*, variation in structural blue color results from variation in the dimensions of components of the keratin matrix as well as variation in larger–scale factors such as the thickness of the feather cortex and the density of barbules (Shawkey et al. 2003a; Shawkey et al. 2005). Moreover, experimentally–induced degradation of the barb cortex and keratin matrix from keratinolytic bacteria results in increases in brightness, hue, and saturation, and a decrease in chroma, of structural blue feathers (Shawkey et al. *In review*). Thus, structural blue colors may be particularly sensitive to damage caused by bacteria and lice.

Our documented changes in hue and chroma of the blue mantle with specimen age may be consistent with some form of parasitic degradation of feather barbs. Such changes in color could also be caused by soiling and feather wear, however, particularly if soiling influences some parts of the reflectance spectrum more than others, as has previously been suggested (Örnborg et al. 2002).

Melanin–based colors are produced by the deposition of melanin pigments into growing feathers (McGraw 2006b). Eumelanins generally absorb light evenly across the bird–visible spectrum, resulting in flat reflectance spectra of low amplitude (Mennill et al. 2003; McGraw 2006b). As such, high concentrations of melanin should result in darker plumage of lower reflectance. As large polymers with strong cross–links to proteins, melanins are thought to confer structural stability to animal and plant tissues, including bird feathers (Burtt 1986; Bonser 1995). Melanized feathers are more resistant to abrasion than unmelanized feathers (Bonser 1995; McGraw 2006b), and recent studies have shown that melanized feathers are less susceptible to the effects of feather chewing lice (Kose et al. 1999) and feather degrading bacteria (Goldstein et al. 2004) than unmelanized feathers. Moreover, melanin pigments are highly biochemically stable (Riley 1997). We therefore expected that, of the three types of color we investigated, melanins would be the least susceptible to long–term degradation (Test 1940). However, we found significant, albeit subtle, variation in the black body plumage of long–tailed manakins. By contrast with crown and mantle coloration, the brightness of the black body plumage increased with increasing specimen age. One possible explanation is that superficial damage to barbs and barbules, be it caused by abrasion or degradation from bacteria or lice, would roughen the surface of the keratin cortex without affecting the

melanin granules themselves, which are found below the cortex. Rougher surfaces increase incoherent scattering of white light (Bradbury and Vehrencamp 1998; Prum 2006), which would thereby increase brightness (Shawkey et al. *In review*). The changes in saturation and chroma of black body plumage are rather more difficult to explain. One possibility is that they result from physical or biochemical changes to other parts of the feather rather than to the melanin granules. Alternatively, eumelanin pigments, which are black, may be more susceptible to degradation than phaeomelanin pigments, which are reddish–brown. Since many melanin–based colors contain both eumelanin and phaeomelanin pigments (McGraw et al. 2004b), a reduced proportion of eumelanin might result in higher reflectance at longer wavelengths and a more brownish appearance (Test 1940). Yet another possibility is that biochemical degradation of eumelanin pigments alters their color.

Using long–tailed manakins as a model system, we have documented differences in plumage reflectance between museum specimens and wild birds for carotenoid, structural, and melanin–based colors. Some degradation appeared to be of a generalized nature, while some changes varied by plumage color type. It is important to note that, in most cases, differences between study skins and wild birds were quite subtle (Fig. 2). Indeed, this is somewhat surprising, given that plumage colors have been shown to change considerably over much shorter time scales (Örnborg et al. 2002; McGraw and Hill 2004). As such, study skins can serve as an accurate representation of the plumage reflectance patterns of wild birds, particularly for broad–scale analyses such as characterizing the reflectance of a particular species or quantifying interspecific differences in color. Intraspecific studies, however, such as assessments of sexual

dichromatism or investigations of geographic variation in ornamentation, should consider the potential effects of specimen age. In most cases, it is possible account for these potentially confounding factors by calculating residuals or using multivariate analyses that include geographic location and specimen age as covariates. Alternatively, one could restrict samples to a particular time period or geographic location if only a few individuals of a particular species are required. We also found that time–dependent plumage color changes varied by plumage type. For example, proportional UV reflectance decreased with specimen age in structural blue plumage but increased with specimen age in red carotenoid plumage. Thus, broad scale studies of particular types of signals based on museum skins, such as investigations of the ubiquity of UV reflectance, should be interpreted cautiously. Finally, our findings highlight the importance of museum practices in maintaining not only the structural integrity of museum skins, but also the quality of their plumage coloration. Minimizing exposure to light, handling, bacteria, fungi, insects, and lice will help to preserve the quality and color of study skins, which, in this era of reduced emphasis on collecting (Remsen 1995; Winker 2004), will allow researchers to benefit from their use for generations to come.

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Table 1. Comparison of plumage reflectance (colorimetric variables) between museum skins and live–caught male long–tailed manakins in definitive plumage. T–tests were used for normally distributed variables and Wilcoxon tests were used when variables departed significantly from normality.

Body region	Color variable	Wild birds (n = 58)	Study skins (n = 55)	Test statistic	P
Red crown	Brightness (%)	12.79 ± 1.92	11.01 ± 2.45	T = 4.31	<0.0001*
	Hue (nm)	632.2 ± 3.74	630.9 ± 4.5	Z = –1.20	0.23
	Saturation	5.62 ± 0.45	4.85 ± 0.60	Z = –6.39	<0.0001*
Blue mantle	Brightness (%)	31.4 ± 3.57	28.9 ± 2.68	T = 4.07	<0.0001*
	Hue (nm)	370.8 ± 9.1	401.5 ± 30.5	Z = 7.54	<0.0001*
	Saturation	0.72 ± 0.09	0.68 ± 0.06	T = –2.12	0.03
Black body	Brightness (%)	3.36 ± 0.91	3.70 ± 0.58	Z = –3.43	0.0006*
	Hue (nm)	588.3 ± 76.4	651.6 ± 80.3	Z = 5.46	<0.0001*
	Saturation	0.47 ± 0.26	0.60 ± 0.15	Z = 5.04	<0.0001*

*Significant after Bonferroni correction

Table 2. Relationship between plumage color variables and specimen age (age = 1 for wild birds) among male long–tailed manakins in definitive adult plumage.

Body region	Color variable	r_s	n	P
Red crown	Brightness (%)	0.12	103	0.41
	Hue (nm)	0.23	102	0.13
	Saturation	0.06	103	0.69
Blue mantle	Brightness (%)	0.28	104	0.06
	Hue (nm)	0.16	104	0.31
	Saturation	0.10	104	0.51
Black body	Brightness (%)	–0.14	103	0.35
	Hue (nm)	0.67	104	<0.0001*
	Saturation	0.15	103	0.32

*Significant after Bonferroni correction

Table 3. Relationship between plumage color variables and geographic location (PC1 location – see methods for details on PCA) among male long–tailed manakins in definitive adult plumage.

Body region	Color variable	r_s	n	P
Red crown	Brightness (%)	–0.38	110	<0.0001*
	Hue (nm)	0.08	109	0.36
	Saturation	–0.30	110	0.001*
Blue mantle	Brightness (%)	–0.34	111	0.0002*
	Hue (nm)	0.35	111	0.0002*
	Saturation	–0.20	111	0.04
Black body	Brightness (%)	0.22	110	0.02
	Hue (nm)	0.17	111	0.07
	Saturation	0.16	110	0.09

*Significant after Bonferroni correction

Table 4. Multiple regression of plumage color variables on specimen age and sampling location (PC1 location – see methods for details on PCA) among male long–tailed manakins in definitive adult plumage.

Body region	Color variable		R^2	F	ß	df	P
Red crown	Brightness (%)	Model	0.26	17.5		2, 100	<0.0001*
		Specimen age		0.21	–0.04	1, 101	0.64
		Location		28.4	–0.49	1, 101	<0.0001*
	Hue (nm)	Model	0.03	1.66		2, 99	0.19
		Specimen age		2.63	–0.17	1, 100	0.11
		Location		1.86	0.14	1, 100	0.18
	Saturation	Model	0.27	18.6		2, 100	<0.0001*
		Specimen age		24.3	–0.45	1, 101	<0.0001*
		Location		2.51	–0.14	1, 101	0.12
Blue mantle	Brightness (%)	Model	0.17	10.1		2, 101	<0.0001*
		Specimen age		3.91	–0.19	1, 102	0.05
		Location		9.29	–0.30	1, 102	0.003*
	Hue (nm)	Model	0.42	36.2		2, 101	<0.0001*
		Specimen age		66.8	0.66	1, 100	<0.0001*
		Location		0.55	–0.06	1, 100	0.46
	Saturation	Model	0.07	4.07		2, 101	0.02
		Specimen age		2.64	–0.17	1, 102	0.11
		Location		2.55	–0.16	1, 102	0.11
Black body	Brightness (%)	Model	0.05	2.41		2, 100	0.09
		Specimen age		3.35	0.19	1, 101	0.07
		Location		0.22	0.05	1, 101	0.64
	Hue (nm)	Model	0.21	13.2		2, 101	<0.0001*
		Specimen age		25.5	0.48	1, 102	<0.0001*
		Location		1.04	–0.10	1, 102	0.31
	Saturation	Model	0.10	5.34		2, 100	0.006
		Specimen age		9.96	0.32	1, 101	0.002*
		Location		0.12	–0.03	1, 101	0.73

*Significant after Bonferroni correction

Table 5. Multiple regression of plumage chroma variables on specimen age and sampling location (PC1 location – see methods for details on PCA) among male long–tailed manakins in definitive adult plumage.

Body region	Color variable		R^2	F	ß	df	P
Red crown	UV chroma	Model	0.39	32.0		2, 100	<0.0001*
		Specimen age		23.2	0.40	1, 101	<0.0001*
		Location		17.8	0.35	1, 101	<0.0001*
	Blue chroma	Model	0.38	30.6		2, 100	<0.0001*
		Specimen age		35.7	0.50	1, 101	<0.0001*
		Location		6.54	0.22	1, 101	0.01
	Green chroma	Model	0.31	22.0		2, 100	<0.0001*
		Specimen age		32.5	0.51	1, 101	<0.0001*
		Location		1.25	0.10	1, 101	0.27
	Red chroma	Model	0.37	29.9		2, 100	<0.0001*
		Specimen age		32.7	–0.48	1, 101	<0.0001*
		Location		7.8	–0.24	1, 101	0.006
Blue mantle	UV chroma	Model	0.39	32.6		2, 101	<0.0001*
		Specimen age		61.3	–0.65	1, 102	<0.0001*
		Location		0.96	0.08	1, 102	0.33
	Blue chroma	Model	0.07	3.84		2, 101	0.02
		Specimen age		7.68	0.29	1, 102	0.007
		Location		0.85	–0.09	1, 102	0.36
	Green chroma	Model	0.30	22.1		2, 101	<0.0001*
		Specimen age		41.5	0.57	1, 102	<0.0001*
		Location		0.60	–0.07	1, 102	0.44
	Red chroma	Model	0.13	7.57		2, 101	0.0009*
		Specimen age		13.4	0.36	1, 102	0.0004*
		Location		0.01	–0.01	1, 102	0.92
Black body	UV chroma	Model	0.20	12.5		2, 100	<0.0001*
		Specimen age		1.42	–0.11	1, 101	0.24
		Location		24.6	0.48	1, 101	<0.0001*
	Blue chroma	Model	0.46	42.7		2, 100	<0.0001*
		Specimen age		39.7	–0.50	1, 101	<0.0001*
		Location		16.3	–0.31	1, 101	<0.0001*
	Green chroma	Model	0.31	22.21		2, 100	<0.0001*
		Specimen age		5.34	–0.21	1, 101	0.03
		Location		25.1	–0.45	1, 101	<0.0001*
	Red chroma	Model	0.71	123.5		2, 100	<0.0001*
		Specimen age		204.7	0.83	1, 101	<0.0001*
		Location		0.93	0.05	1, 101	0.34

*Significant after Bonferroni correction

FIGURE CAPTIONS

Figure 1. Map of Central America showing geographic distribution of long–tailed manakins, *Chiroxiphia linearis*. The magnitude of location PC1 scores is also shown.

Figure 2. Average reflectance spectra for the red crown, blue mantle, and black body among male long–tailed manakins in definitive adult plumage. Solid lines represent a subset of live–caught males (n = 15) and dashed lines represent a subset of study skins (n = 15).

Figure 3. Box plots showing differences in plumage color variables between live–caught male long–tailed manakins in definitive adult plumage and study skins. Horizontal lines in box plots show 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles. Asterisk identifies differences that are statistically significant after Bonferroni correction (see Table 1).

Figure 4. Scatterplots showing relationship between plumage color variables and residual specimen age (controlling for geographic location) among male long–tailed manakins in definitive adult plumage. Regression lines are included in plots that show a statistically significant correlation after Bonferroni correction (see Table 4).

Figure 5. Scatterplots showing relationship between plumage color variables and residual geographic location (controlling for specimen age) among male long–tailed manakins in

definitive adult plumage. Regression lines are included in plots that show a statistically significant correlation after Bonferroni correction (see Table 4).

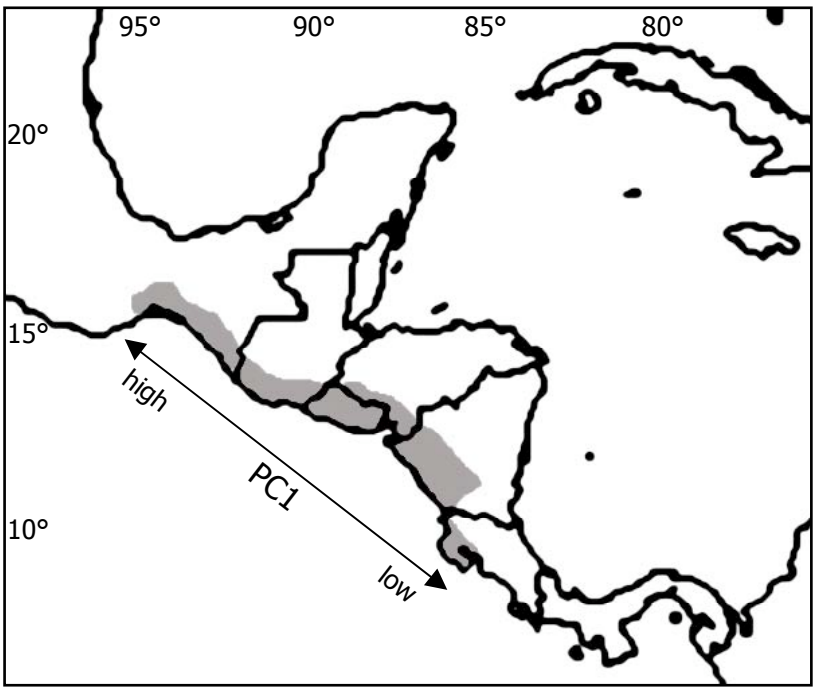

Figure 1

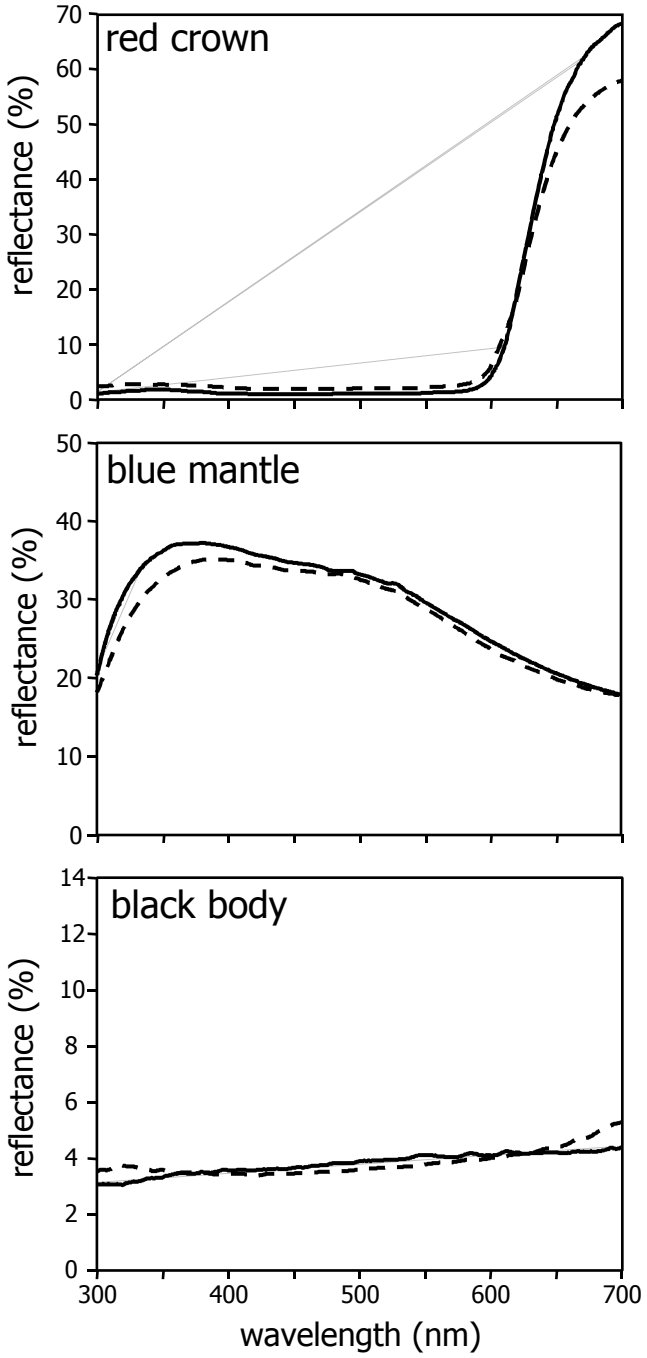
red crown
blue mantle
black body
reflectance (%)
reflectance (%)
reflectance (%)
wavelength (nm)
0
10
20
30
40
50
60
70
0
10
20
30
40
50
0
2
4
6
8
10
12
14
300
400
500
600
700

Figure 2

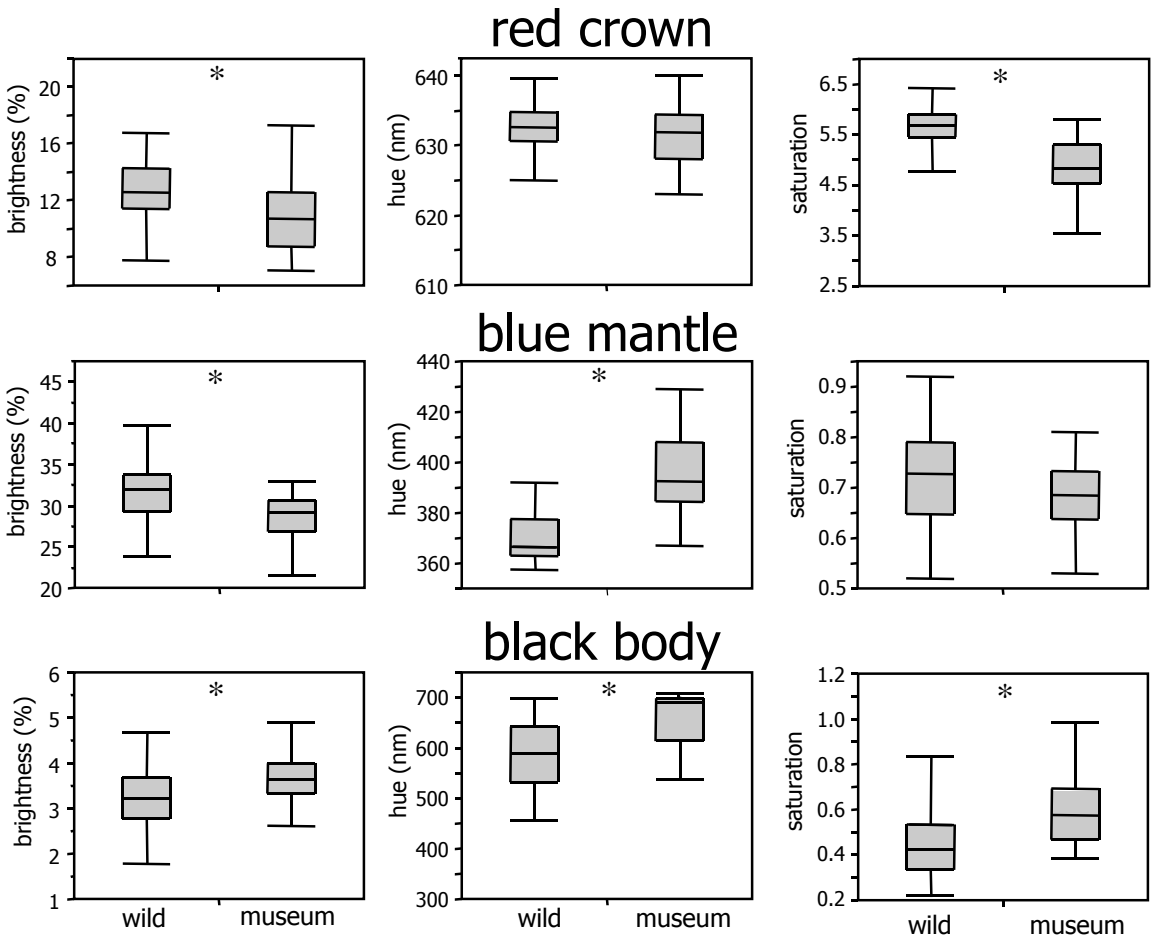
red crown
blue mantle
black body
brightness (%)
hue (nm)
saturation
wild
museum
*

Figure 3

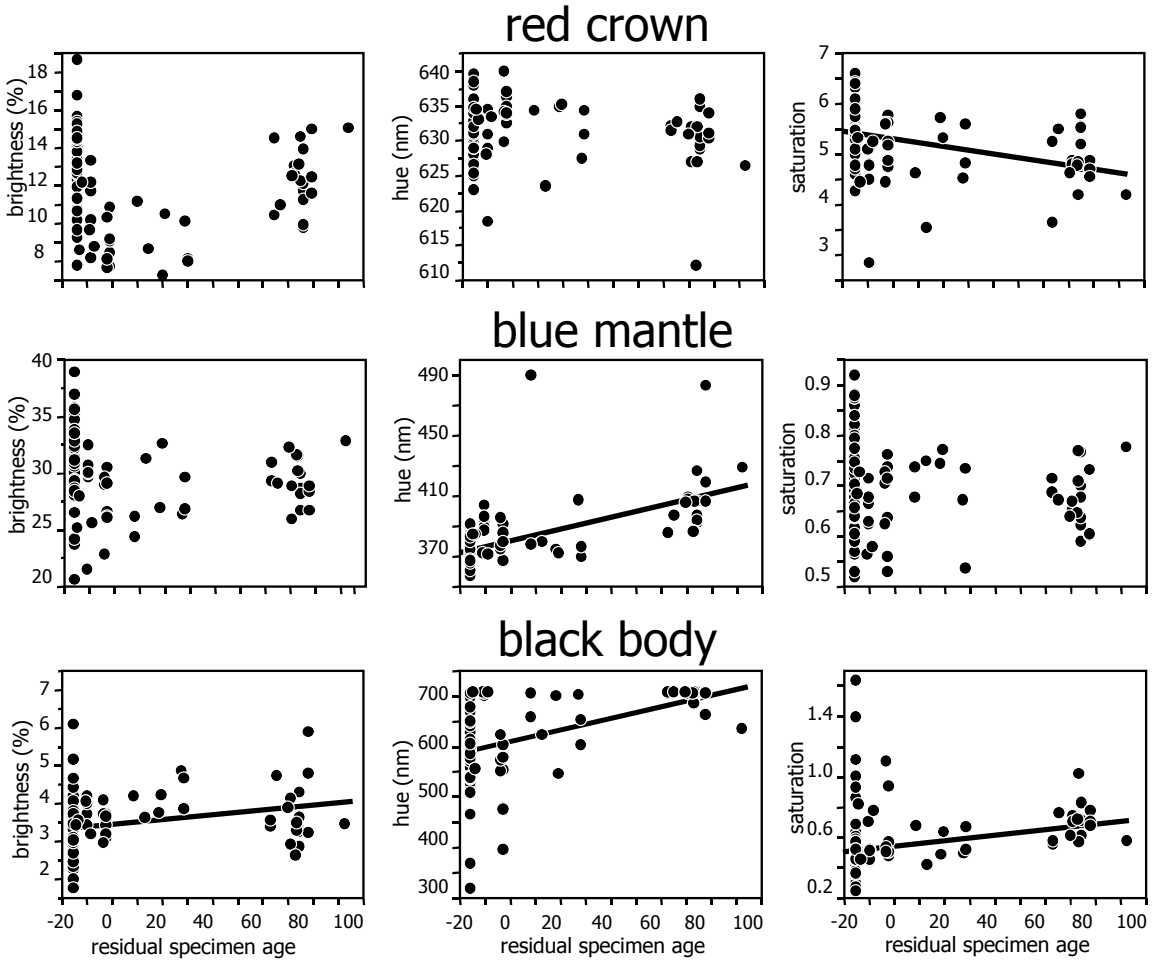
red crown
blue mantle
black body
brightness (%)
hue (nm)
saturation
residual specimen age

Figure 4

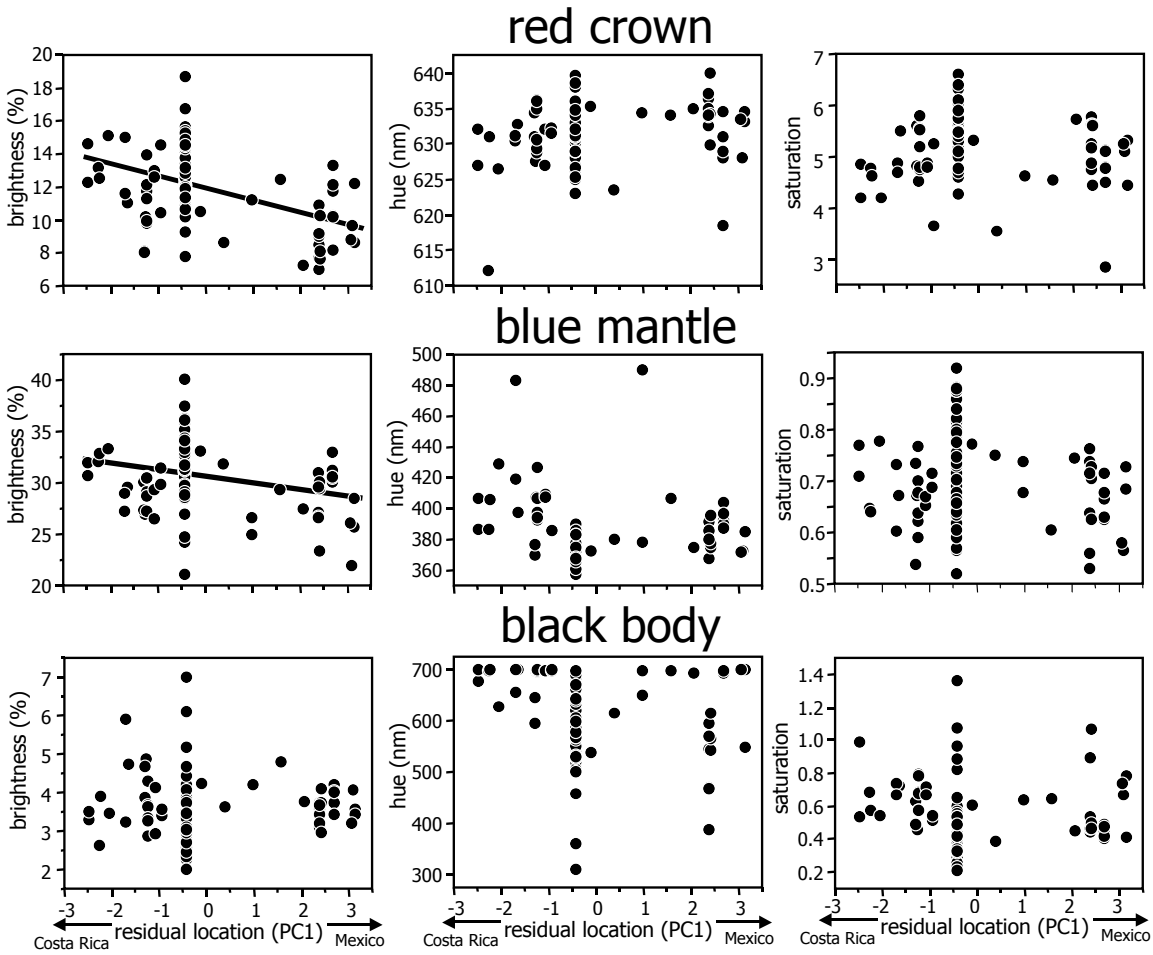
red crown
blue mantle
black body
brightness (%)
hue (nm)
saturation
Costa Rica
residual location (PC1)
Mexico

Figure 5

CONCLUSIONS

As a consequence of their lek-based mating system, manakins have proven to be an excellent system for investigating the influence of natural and sexual selection on plumage coloration and morphology. First, I demonstrated that young male long–tailed manakins, *Chiroxiphia linearis*, exhibit remarkably age–specific patterns of plumage coloration before attaining definitive adult plumage in their fifth year. This unusual pattern of delayed plumage maturation likely serves an important function in maintaining age–graded dominance hierarchies and mating queues at lek sites. Second, I found that patterns of sexual dimorphism within *C. linearis* and among *Chiroxiphia* manakins are consistent with predictions of natural and sexual selection hypotheses. Sexual selection for acrobatic display efficiency appears to act strongly on males, whereas natural selection through intersexual competition and reproductive role division appears to constrain dimorphism in some traits while enhancing it in others. Third, I used irradiance and reflectance spectrometry and avian perceptual models to quantify the degree of conspicuousness in male plumage and crypsis in female plumage of long–tailed manakins. Males did not appear to adjust the timing or location of their displays to enhance conspicuousness, but rather displayed in the most common light environment of forest shade. Displaying this light environment may reflect a trade–off between maximizing conspicuousness in males while minimizing it in females. Finally, I found significant differences in plumage coloration between museum specimens and wild birds.

The magnitude and direction of these differences depended in part of the mechanism of color production. Most differences were relatively subtle, however, and my findings justify the use of museum specimens to assess plumage coloration for various types of studies.