Structural plumage colour and parasites in satin bowerbirds 
*Ptilonorhynchus violaceus*: implications for sexual selection

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We investigated whether variation in structural plumage coloration in satin bowerbirds, *Ptilonorhynchus violaceus*, could reveal the intensity of infection from parasites, as predicted from models of parasite-mediated sexual selection (PMSS). To do this, we captured adult male, female, and juvenile male satin bowerbirds in Queensland, Australia, and objectively measured individual plumage reflectance from four body regions using a spectrometer. We quantified both ectoparasite load and the intensity of infection from blood parasites. In iridescent blue adult males, plumage reflectance is unimodal, with a single peak in the ultraviolet, while in greenish females and juveniles, plumage reflectance is bimodal, with peaks in both the ultraviolet and green portions of the spectrum. In adult males, the intensity of infection from blood parasites was best predicted by plumage brightness (total reflectance), with brighter males having fewer parasites. Similarly, juvenile males exhibiting greater UV chroma (proportion of reflectance in the UV) had fewer blood parasites. Our findings support a key prediction of PMSS models and provide the first evidence that a structural colour ornament can signal the intensity of infection from blood parasites.

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Females may prefer elaborate male ornaments, despite obvious costs to the bearer, if these ornaments reveal aspects of male quality, such as reduced parasite loads (Hamilton and Zuk 1982). By mating with parasite-free males, females may gain direct benefits (e.g., avoidance of parasite transmission, quality of paternal care; Clayton 1991) and/or indirect benefits (parasite resistance genes for their offspring; Hamilton and Zuk 1982). Many investigations of parasite-mediated sexual selection (PMSS) have justifiably focused on the plumage coloration of birds, as this is a conspicuous visual signal that can be objectively quantified (see Endler and Lyles 1989) and is directly involved in mate choice in several species (reviewed in Andersson 1994).

To date, intraspecific investigations of PMSS have disproportionately involved carotenoid- and, to some extent, melanin-based signaling systems (reviewed in Møller et al. 1999). Recent comparative and intraspecific studies have, however, highlighted the important role that structural plumage coloration may play in sexual selection (e.g., Andersson and Amundsen 1997, Hunt et al. 1998, Owens and Hartley 1998, Keyser and Hill 2000). Indeed, structural colours have the potential to reveal different kinds of information about the signaler since they are produced by feather microstructure rather than pigmentation (e.g., McGraw et al. 2002).

In this study we investigated PMSS in satin bowerbirds, *Ptilonorhynchus violaceus*. We first characterized variation in the plumage reflectance and parasite infection intensities in adult males, females, and juveniles, then assessed the potential for their structural plumage coloration to serve as an indicator of the intensity of infection from parasites. We thus predicted a negative relation between parasite loads and the quality of plumage colour among individuals. Borgia and Collis (1989) previously reported a negative relationship between ectoparasite load and mating success in satin bowerbirds. They also attempted to score plumage col-
oration visually but they could find no consistent differences between males (Borgia and Collis 1989), which is not surprising given the limitations of short-wave human vision compared to that of birds (Cuthill et al. 2000). Borgia and Collis (1989, 1990) proposed that their data favored a parasite avoidance model of PMSS in satin bowerbirds. Our findings suggest that, at least for haematozoan parasites, a different sexual selection mechanism might be operating.

Methods
We studied a population of satin bowerbirds throughout their breeding season, from September to December 2000, in Mount Baldy State Forest, near Atherton (17°30' S, 145°30' E), Queensland, Australia. The distribution of this subspecies, *P. v. minor*, is limited to the highlands of northeastern Queensland (Pizzey and Knight 1997). Using mist-nets baited with blue objects, we captured 11 adult males, 9 juvenile males, and 6 females and fitted each bird with a unique combination of one stainless steel band and two colour bands. We initially sexed green birds using plumage characteristics (Vellenga 1980), and confirmed sex assignments by collecting a small blood sample from each individual and performing molecular analysis using sex-specific DNA primers (Griffiths et al. 1998).

Plumage colour
We measured plumage reflectance of four body regions (wing coverts, rump, mantle, breast) on each individual, using an S2000 spectrometer and PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, Florida, USA). Measurements were taken perpendicular to the feather surface. All reflectance readings were expressed as the proportion of reflectance from a Spectralon® white standard, an almost perfect reflector. We obtained five reflectance spectra for each region, moving the probe at least 5 mm between readings. We used an average spectrum from the five readings for each body region in the following analyses.

UV/blue colour variables
To facilitate sex-, age-, and parasite-based comparisons of plumage reflectance, we calculated colour variables according to three dimensions of colour vision: hue, brightness, and chroma. For adult males, reflectance spectra are unimodal, with a peak in the UV region (Fig. 1). To estimate hue, we used the wavelength corresponding to the maximum reflectance in the range of spectral sensitivity in birds (300–700 nm; Cuthill et al. 2000). We calculated total brightness as the mean reflectance over this range, and UV chroma (a measure of spectral saturation) as the proportion of total reflectance occurring in the UV region.

UV/green colour variables
In contrast to adult males, reflectance spectra for the greenish plumage of females and juvenile males are best described as bimodal, with peak reflectance values in the UV/blue (300–450 nm) and green/yellow (450–700 nm) regions (Fig. 1). Thus, we calculated both ‘blue’ and ‘green’ hues for females and juvenile males as the wavelength of maximum reflectance in each of those two regions of the spectrum, respectively, and total brightness as described for adult males. We calculated UV chroma as described above and green chroma as the proportion of total reflectance in the green region, from 512 to 575 nm. We selected wavelength ranges for these chroma computations by considering the intersect-

Fig. 1. Plumage reflectance spectra from four different body regions in adult and juvenile male satin bowerbirds. These are mean reflectance curves from individual males chosen because their brightness, chroma, and hue values were representative of the means for each of their plumage types. Female spectral reflectance is similar to that of juvenile males except for the breast regions (see text). Note the different scales on the reflectance axes.

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Assessing parasite infection

To assess ectoparasite load, we counted the number of *Myrsidea ptilonorhynchi* lice around the head and eyes of the birds we captured. These lice presumably aggregate in these body regions because they can’t easily be preened away from those areas. This species of louse belongs to a suborder (Amblycera) of blood-feeding lice known to influence the fitness of their hosts (Clayton 1991) and appears to be the only common ectoparasite on satin bowerbirds (Borgia and Collis 1989, 1990).

To assess the intensity of infection from blood parasites, we collected a small blood sample from each bird by piercing the brachial vein, drawing blood into a capillary tube, and thinly smearing it onto a glass slide. We fixed and stained the slides using the Hema 3™ staining procedure (Fisher Scientific). All slides were scored by the same observer, blind to the identity of the bird being scored. We observed the stained slides under oil immersion at 1250 × magnification, scanning each slide for haemosporidian parasites until approximately 10,000 red blood cells had been surveyed. The only common parasite we encountered was *Haemoproteus* sp. in the form of extra-erythrocytic, developing, and mature gametocytes (Campbell 1988). Thus, we restricted our blood parasite analyses to the total number of mature *Haemoproteus* parasites per 10,000 red blood cells. Neither ectoparasite load ($r = 0.26, n = 26, P = 0.19$) nor the intensity of infection from blood parasites ($r = 0.06, n = 26, P = 0.76$) varied significantly with date of sampling.

Results

Plumage colour in satin bowerbirds

The UV/blue hue of adult males peaked at significantly shorter wavelengths than that of both females and juvenile males (Tukey-Kramer post hoc tests, $P < 0.05$), which were not significantly different from each other (Table 1). There were also significant differences in both the total brightness and UV chroma of adult males compared to females and juvenile males (Tukey-Kramer test, $P < 0.05$), with females and juveniles exhibiting greater overall reflectance but relatively less reflectance in the UV region (Table 1). The green chroma, UV chroma, and green hue of juvenile males and females were remarkably similar (Table 1).

In satin bowerbirds, juvenile males retain a cryptic green plumage, similar to that of females, until they reach six years of age, when they moult into the iridescent blue characteristic of adult males (Vellenga 1980). The breast plumage of juvenile males has been described as more greenish than that of females (Vellenga 1980) and females indeed had significantly brighter (i.e., paler green) breasts than juvenile males (ANOVA, $F_{1,14} = 8.70, P = 0.01$).

Parasite infection

The prevalence of ectoparasitic infection was 91%, 100% and 89% among adult males, females, and juvenile males, respectively, with significant differences in the intensity of ectoparasitic infection among these age/sex categories (ANOVA, $F_{2,23} = 3.63, P = 0.04$; Fig. 2). Adult males had significantly larger ectoparasite loads.
loads than juvenile males (Tukey-Kramer test, $P < 0.05$), but there were no significant differences between females and either adult males or juveniles ($P > 0.05$).

Based on an assessment of 10,000 red blood cells, the prevalence of infection from blood parasites was 64%, 100% and 78% among adult males, females, and juvenile males, respectively. However, a more detailed inspection of the blood smears of the six individuals that scored zero in this analysis revealed that they had all been exposed to blood parasites; four were infected with at least one mature *Haemoproteus* and two carried gametocytes. There were no significant differences in the intensities of blood parasite infections among the age/sex categories (ANOVA, $F_{2,23} = 1.10$, $P = 0.35$, Fig. 2). There was also no relationship between ectoparasite load and the intensity of infection from blood parasites among all birds ($r = 0.009$, $N = 26$, $P = 0.96$) or when each age/sex category was considered separately ($P = 0.60$, $P = 0.15$, $P = 0.73$ for adult males, females, and juveniles, respectively).

**Can plumage colour reveal the intensity of infection from parasites?**

To determine which plumage characteristics could best reveal the intensity of infection from parasites, we constructed multiple regression models using a backward stepwise procedure. For adult males, we constructed two models, one for each type of parasite, with parasite load as the dependent variable and mean total brightness, UV chroma, and UV/blue hue as potential predictor variables. In adult males, total plumage brightness was the only significant predictor of the intensity of infection from blood parasites, explaining over 35% of the variation in infection intensity (standardized beta $= -0.67$, $P = 0.03$; $P > 0.1$ for all other variables). Thus, brighter adult males had fewer blood parasites (Fig. 3). None of the plumage variables were significant predictors of ectoparasite load ($P > 0.15$).

For juvenile males, we included green hue, UV/blue hue, total brightness, UV chroma, and green chroma as potential predictor variables. UV chroma was the only significant predictor of the intensity of infection from blood parasites, explaining over 50% of the variation in infection intensity (standardized beta $= -0.71$, $P = 0.03$; $P > 0.15$ for all other variables). Thus juvenile males whose plumage exhibited greater UV saturation were infected with fewer blood parasites (Fig. 3). None
of the plumage variables were significant predictors of ectoparasite load in juvenile males (P > 0.5). The small number of females sampled did not allow us to perform multivariate analyses while including the five plumage variables measured.

**Discussion**

As we have shown here, there is considerable UV reflectance in both the iridescent blue plumage of adult male satin bowerbirds and the greenish plumage of females and juvenile males. The coefficients of variation (CV) for the structural plumage colour variables of adult males tended to be higher than those of females (Table 1), as would be predicted for traits under the influence of sexual selection and the CVs also tended to be higher for UV/blue than for green colour variables. The iridescent plumage colour of males is produced within the barbules by constructive interference from arrays of melanin granules and/or air vacuoles suspended in barbule keratin (Fox 1976, Prum 1999). In contrast, the greenish appearance of females and juvenile males likely results from a combination of coherently scattered light waves in the spongy, medullary layer of feather barbs and carotenoid pigmentation (Fox 1976, Prum 1999, S. Doucet pers. obs.). The UV/blue peak in adult male reflectance spectra occurred at shorter wavelengths than that of females and juveniles and coincides with the peak sensitivity of UV-sensitive cones in passerine birds (Cuthill et al. 2000). This difference between reflectance spectra in the UV range may result from the different production mechanisms for these two types of structural colour. Overall, the plumage coloration of females and juvenile males was similar; the only detectable difference was revealed in the brighter breasts of females.

Ectoparasite prevalence was high among all satin bowerbirds, but adult males had significantly more ectoparasites than either females or juveniles. These results provide an interesting contrast to the findings of Borgia and Collis (1990) who showed that, in three consecutive years, adult males had fewer ectoparasites than females and juveniles in the southern subspecies of satin bowerbirds, *P. v. violaceus*. A number of different factors may account for this variation in ectoparasite intensities, including considerable climate and habitat differences between the two subspecies distributions.

There were no significant differences between sex and age classes in the intensity of infection from blood parasites, possibly resulting from the high individual variation in infection intensities (range 0–196 *Haemoproteus* parasites per 10,000 red blood cells). We found no evidence of a correlation between degrees of infection from ecto- and endoparasites. The lack of a relationship between lice and blood parasites may reflect their different transmission modes or the different selective pressures influencing susceptibility to these parasites.

Our findings support an important intraspecific prediction of PMSS models: that within species, there should be a negative association between parasite load and male showiness (e.g., Hamilton and Zuk 1982). In adult males, total plumage brightness was a significant predictor of the intensity of infection from blood parasites, as was UV chroma in juvenile males. Because satin bowerbirds are long-lived, and the birds in our study were all exposed to blood parasites, low parasite intensities may be related to parasite resistance in this species.

Female satin bowerbirds make multiple courtship visits to several males, eventually narrowing down the pool of copulation partners to one or two males (Uy et al. 2000). Choosiness is therefore likely to incur some costs to females in terms of time and energy constraints (Gibson and Langen 1996) and the increased likelihood of aggressive encounters and forced copulations with other males (Uy et al. 2000). Yet, male satin bowerbirds provide only gametes to females, suggesting that female choosiness may be driven by the potential to gain indirect fitness benefits. Our findings suggest that females could choose relatively parasite-free males by assessing male plumage colour, and as a result acquire parasite-resistance genes for their offspring, if parasite resistance is heritable (Hamilton and Zuk 1982).

Obtaining parasite resistance for offspring is of no small consequence. Although much of the evidence emphasizing the pathogenicity of *Haemoproteus* parasites originates from laboratory studies of domesticated birds, reports of dead or moribund wild birds with acute haemosporidian infections generally support laboratory findings (Atkinson and van Riper 1991). In blue tits *Parus caeruleus*, females medicated with an antimalarial substance exhibited a reduced level of *Haemoproteus* infection, and had fewer nestling deaths and greater fledging success than unmedicated control females (Merino et al. 2000). Given that younger birds are more susceptible to infections (Scott and Edman 1991) and more likely to die from acute haematozoan infections (Atkinson and van Riper 1991), the consequences of acquired genetic resistance could be even more pronounced in nestlings. Thus, choosing parasite-resistant copulation partners could thus influence lifetime reproductive success in female satin bowerbirds by impacting offspring survival.

This is the first study to show an association between structural plumage colour and blood parasites (see also Doucet and Montgomerie 2003), although a link between structural plumage colour and feather mites has been documented (Harper 1999). In our study, neither adult male plumage nor juvenile plumage was a significant predictor of ectoparasite load. This finding may not be surprising, given that ectoparasite load likely
varies on a shorter time scale than chronic infection from blood parasites. Thus, ectoparasite load is more likely to signal current condition. Indeed, we show elsewhere that males with fewer ectoparasites build higher quality bowers, another trait likely to be influenced by current condition (Doucet and Montgomerie 2003). As Borgia and Collis (1989, 1990) remarked, ectoparasites are quite visible in satin bowerbirds, and the fact that females avoid mating with highly parasitized males suggests that these ectoparasites may fall under a ‘parasite avoidance’ rather than ‘good genes’ model of parasite-mediated sexual selection.

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