J Ornithol
DOI 10.1007/s10336-012-0855-x

ORIGINAL ARTICLE

The number and coloration of white flank spots predict the strength of a cutaneous immune response in female Diamond Firetails, *Stagonopleura guttata*

Valeria Zanollo · Matteo Griggio · Jeremy Robertson · Sonia Kleindorfer

Received: 20 January 2012 / Revised: 11 April 2012 / Accepted: 23 April 2012

Abstract Colour variation in birds is often used to signal functional differences between individuals and sexes, though white plumage has usually been disregarded because white feathers were thought to be cheap to produce and hence unreliable signals. Here, we provide evidence for sex-specific morphological and colour differences in the strikingly patterned but apparently monomorphic Diamond Firetail (*Stagonopleura guttata*). We found that males had longer and darker tails, wider lores and a darker bill, while females had significantly more white flank spots. These gender-specific trait differences could be used to signal individual quality in sexual or social interactions, and hence we examined the correlation between these traits and the cell-mediated immune response (phytohaemagglutinin, PHA test). During both the breeding and non-breeding seasons, we found a positive correlation between PHA response and white flank spot number as well as their ultra-violet reflectance in females, and a negative correlation with white chroma and hue. In males, there was a positive correlation between PHA response and red rump reflectance (hue, red-chroma). There was no association between PHA response and either tail length or lore depth in either sex. These results add to a growing body of evidence that female spot number and white plumage reflectance signal quality.

Keywords Sexual dimorphism · Honest signals · White plumage · Spottiness · Female quality · PHA test

Communicated by L. Fusani.

V. Zanollo · J. Robertson · S. Kleindorfer
School of Biological Sciences, Flinders University of South Australia, Adelaide, SA, Australia

M. Griggio (✉)
Department of Integrative Biology and Evolution, Konrad Lorenz Institute of Ethology, University of Veterinary Medicine Vienna, Savoyenstraße 1a, 1160 Vienna, Austria
e-mail: matteo.griggio@vetmeduni.ac.at

Zusammenfassung

Anzahl und Farbton der weißen Seitenflecken als Indikator der kutikulären Immunantwort von Diamantfinken (*Stagonopleura guttata*)

In vielen Vogelarten dienen Farbmerkmale dazu funktionale Unterschiede zwischen Individuen und Geschlechtern zu signalisieren. Weiße Gefiederfarben sind in diesem Kontext selten untersucht worden, da angenommen wird, dass die Produktion weißer Federn mit geringen Kosten verbunden ist und somit ein unzuverlässiges Signal darstellt. In der vorliegenden Studie beschreiben wir geschlechtsspezifische Unterschiede in Morphologie und Färbung des auffallend bunten, sexuell monomorphen Diamantfinken (*Stagonopleura guttata*). Männchen besaßen längere und dunklere Schanzfedern, breitere dunkle Streifen zwischen Auge und Schnabel und einen dunkleren Schnabel, während Weibchen eine signifikant größere Anzahl weißer Seitenflecken aufwiesen. Da diese Merkmale in sexuellen oder sozialen Interaktionen genutzt werden können untersuchten wir den Zusammenhang zwischen ihnen und der zellbasierten Immunantwort (Phytohaemagglutinin, PHA test). Sowohl innerhalb als auch außerhalb der Brutsaison fanden wir eine positive Korrelation zwischen der PHA-Antwort und der Anzahl der weißen Flecken sowie der UV-Färbung der Weibchen. Die Korrelation mit Farbsättigung und Farbton der weißen Flecken war signifikant negativ. Bei den Männchen zeigte

Published online: 12 May 2012

sich eine positive Korrelation zwischen PHA-Antwort und roter Färbung (Farbsättigung und Farbton) des Bürzels. In beiden Geschlechtern fanden wir keinen Zusammenhang zwischen der Länge der Schwanzfedern oder der Breite des Kopfstreifens und der PHA-Antwort. Diese Ergebnisse erweitern unser Wissen über den Gebrauch von Fleckenmuster und weißer Gefiederfarbe als weibliches Qualitätsmerkmal.

Introduction

Colour variation is a widespread phenomenon in birds and its evolution and adaptive function has been hotly debated since Darwin (reviewed in Cronin 1991). Colour variation is often correlated with sex and age (Butcher and Rohwer 1989), and hence birds may use visual cues to recognise the sex and maturity of conspecifics. Colour may also signal functional differences in quality that can be used to discriminate between potential partners and rivals, and hence optimise mate choice (McGraw and Ardia 2003; Bortolotti et al. 2006; Senar 2006, Amundsen and Pärn 2006; Clutton-Brock 2007). Curiously, despite the apparent advantages of having colour signals that can be used to discriminate between the sexes, over 50 % of bird species are monomorphic in appearance (Griffiths et al. 1998; Heinsohn et al. 2005). Yet this apparent similarity in the sexes may be deceptive because birds have a broader visual spectrum and can detect cues invisible to humans (Cuthill et al. 2000; Eaton 2005). Hence, species of birds that appear monomorphic to humans may well be dimorphic to their conspecifics, and use subtle variations in their plumage as both species-specific signals and for communication within and between the sexes.

Colour variation may also be a reliable indicator of the general health of an individual (Jawor and Breitwisch 2003; Hill and Montgomerie 1994), whereby brighter plumage and integumentary colour (bill, legs, skin and comb) have been correlated with better nutritional condition (Doucet 2002; Griffith and Pryke 2006), fewer parasites (Zuk et al. 1990; McGraw and Hill 2000; Mougeot et al. 2005) or a stronger immune response (Linvelle and Breitwisch 1997; Saks et al. 2003; McGraw et al. 2006; Jacquin et al. 2011). Most research on feather colouration has concentrated on melanin-based ornaments (Evans et al. 2000; Griggio and Hoi 2010; for a review, see McGraw 2006), carotenoid-based ornaments (Lonzano 1994; Hill 2002; Griggio et al. 2009a; for a review, see Hill 2006), and structural colouration (Doucet 2002; Siefferman and Hill 2005; Griggio et al. 2009b, 2010). In contrast, white plumage patches have been little studied even though they are present in many birds with different grades of expression and conspicuousness. In part, white patches have been little researched because the keratin of unpigmented feathers is structurally achromatic and such ornaments were considered cheap to produce (Török et al. 2003; Prum 2006). However, there is growing evidence that white patches have a cost and therefore a potential function as an honest signal (Höglund and Lundberg 1987; Pärt and Qvarnström 1997; Kose and Møller 1999; Kilpimaa et al. 2004; McGlothlin et al. 2007; Griggio et al. 2011). For example, white feathers are costly in Barn Swallows (*Hirundo rustica*) because feather lice are more frequent on feathers without melanin and these unpigmented feathers are more likely to break (Kose and Møller 1999). Similarly, experimentally manipulating the diet of Dark-eyed Juncos (*Junco hyemalis*) has shown that they develop larger and brighter white patches when they have a high-protein diet (McGlothlin et al. 2007). Hence, in at least some species, it is clear that white plumage patches are costly to produce and maintain and could function as reliable signals of phenotypic quality.

Moreover, the majority of studies investigating the cost and possible function of plumage ornaments have largely focused on male ornaments, and there has been little research into females, especially in species in which the sexes are apparently equally ornamented (reviewed in Clutton-Brock 2009). In addition, there are only a few recent studies investigating the role of a white plumage patches as indicators of female quality (Velando et al. 2001; Potti and Merino 1996; Morales et al. 2007; Hanssen et al. 2009).

Our study species is the Diamond Firetail (*Stagonopleura guttata*), an endemic Australian estrildid finch that is notoriously difficult to sex in the hand (Higgins et al. 2006). Both sexes have a conspicuous plumage characterised by a red bill, black lores and breast band. The species derives its name from the conspicuous red rump and a striking pattern of white spots (diamonds) on black flanks. The carotenoid-based hue of the bill is a responsive and reliable short-term indicator of carotenoid concentration that supplements the longer-term signalling of the feathers (Stirnemann et al. 2009). We have previously shown that the number of white flank spots varies between individuals and is correlated with female (but not male) social status and dominance during feeding contests (Crowhurst et al. 2012). Exposure of the white spots also varies with behavioural context (resting, feeding, displaying) and with the intensity of behavioural interactions (Dunbar 2008; personal observation). The aim of this study is to identify which sexually dimorphic traits could be an expression of individual condition. First, we identify morphological or colour traits that are dimorphic; and second, we compare individual variation in these traits with immune activity, measured as the skin swelling to a phytohaemagglutinin

(PHA) test; this skin reaction is thought to provide information about the cell-mediated immune response (Tella et al. 2008). For the PHA response, we focus our analyses in particular on the role of variation in white spot number for response intensity.

Methods

Study species

The Diamond Firetail is a small (~17 g) estrildid finch that occurs in woodlands and open forest of temperate, semi-arid and arid areas of south–eastern Australia (Higgins et al. 2006). It is primarily granivorous and may consume the seeds of introduced grasses that have replaced native plants in much of its range (Read 1994). Since the 1980s, habitat loss has drastically reduced Diamond Firetail populations, and it is now nationally listed as near threatened (Garnett and Crowley 2000; Ford et al. 2001; Garnett et al. 2011).

Few field studies are available, and little is known about Diamond Firetail breeding or morphological variation in the wild (O'Gorman 1980; Higgins et al. 2006; McGuire and Kleindorfer 2007). Most of what is known about Diamond Firetails is based on observations by aviculturists (O'Gorman 1980, 1981; Higgins et al. 2006). The finches are socially monogamous and breed in pairs from August to February (O'Gorman 1981). The male courts the female with an elaborate display that usually involves bobbing on a branch while holding a piece of green grass. Both adults usually build the bottle-shaped nest, and females lay 3–7, but usually 4–5, eggs (Higgins et al. 2006). Both parents incubate and feed nestlings and fledglings (Higgins et al. 2006). Juveniles acquire their adult plumage at ~10–14 weeks in warmer climates and ~16–20 weeks in cooler climates; males acquire their mature plumage earlier than females (O'Gorman 1980). Both sexes have red rump feathers, black lores, black breast bands and, usually, >20 white flank spots (Higgins et al. 2006). Aviculturists assess the sex of the birds using bill colouration during the breeding season when male bills are described as "almost purple" and female bills as "coral pink" (O'Gorman 1980; Higgins et al. 2006).

Morphological measurements

We measured morphological features of captive finches: 36 males and 33 females at the Adelaide Zoo in 2006, 2007, and 2009, and 42 males and 36 females in aviaries at Flinders University between 2009 and 2011. We also measured wild finches: 14 males and 17 females caught at Monarto Zoological Park (35°8′S, 139°8′E) and 10 males and 6 females at Sandy Creek Conservation Park (34°36′S, 138°51′E) in South Australia between 2004 and 2009. Thus, the total sample size was 102 males and 92 females. We measured six morphological traits using a digital calliper (Mitutoyo, Japan) to the nearest 0.1 mm: (1) bill length (bill tip to the base of feathers); (2) flattened wing length; (3) tail length (middle tail feather); (4) tarsus length; (5) breast band width (measured at rest, with the bird in an upright position); and (6) left and right lore width. We also measured the mass (g) with an electronic scale (EEA digital Scale, T200; Diamond Systems) to the nearest 0.1 g. All measurements were made by S.K. to eliminate measurement variation between researchers. Each bird was individually marked with an Australian Bird and Bat Banding Scheme alloy identification band and a unique combination of plastic colour bands.

Bill and plumage colouration

We measured bill and feather colouration from 62 birds (31 males, 31 females) from the Flinders University captive population in October 2010. We decided to examine sex differences during the breeding season when they are most apparent, and when these differences have most commonly been observed by aviculturists. We measured colour variables from four body regions: bill (red), rump feathers (red), and left flank spots and right flank spots (white). We measured the entire colour range (300–700 nm) using an Ocean Optic USB4000 spectrometer connected to a bifurcated fibre optic probe and an Ocean Optics LS-1 tungsten halogen light source. All the colour measurements were done on the same day, in a closed room with artificial dimmed light, so that the ambient light in the room was constant through all the measurements. Measurements were calibrated using a 99 % diffuse white-standard reference tile (WS-2) subtracting the black current (electromagnetic disturbance) from the spectrum using SpectraWin software (Top Sensor Systems, Eerbeek, Netherlands). Calibration was performed before each individual. For each bird, we took three measurements for each of the four regions, removing the probe between each measurement and averaging the three scores per area. Readings were measured with a black reference pad beneath the feathers to exclude light reflectance from alternate sources; the probe, including a probe tip to reduce ambient light interference, was held at 90° and 2 mm above the focal feather. From the resulting reflectance spectra, we quantified colour using five main standard indices: (1) hue; (2) brightness; (3) chroma; (4) red-chroma; and (5) UV-chroma (Montgomerie 2006). Mean brightness was calculated as the mean summed reflectance ($R_{300–700\ nm}$). Hue (λ_{R50}) was calculated as the wavelength halfway between the highest reflectance point and the lowest reflectance point

[$\lambda_{R50} = (\lambda_{Rmax}-\lambda_{Rmin})/2$]. Chroma was calculated as the difference between the highest and lowest reflectance divided by the average reflectance [$(R_{max}-R_{min})/R_{average}$]. UV-chroma was calculated as the proportion of the UV reflectance on total reflectance ($R_{300–400\ nm}/R_{300–700\ nm}$). Red-chroma was calculated as the percentages of total light reflected in the range 575–700 nm.

Spot number

Flank spot numbers on the left and the right sides of 161 individuals (84 males, 77 females) were counted at the time of banding, both from the two captive populations (Flinders University and Adelaide Zoo) and the wild populations (Monarto Zoological Park and Sandy Creek Conservation Park). To assess the possible effect of age on spot number, we compared spot number in 22 males and 14 females that were measured in 2009 and 2010 (of these, we also have measurements of 9 males and 6 females in 2011) at Flinders University.

Molecular sexing

Birds were genetically sexed. A blood sample of 0.01 ml was collected from each bird by jugular venipuncture using a 0.5-ml syringe (29 G½″, 0.33 × 12.7 mm) (Campbell 1995). The blood sample was stored on FTA paper (Whatman International, Cambridge, UK) and the sex was identified using molecular markers (DNA Solutions Laboratory, Melbourne) (Griffiths et al. 1998).

PHA response in captivity

The phytohaemmaglutin (PHA) response was used to estimate the T cell-mediated immune response (Salvante 2006; Tella et al. 2008). This test is commonly used in captive and wild birds (Kennedy and Nager 2006; Ardia and Schat 2008). Nevertheless, we are aware that the use of the PHA test as a measure of the activation of the cellular immune system has been questioned for many years, and some studies suggest that the reaction caused by the injection could just be a non-specific inflammatory response without T cell involvement (Martin et al. 2006; Sarv and Hõrak 2009; Vinkler et al. 2012). Aware of these reservations, but in line with many studies that use the PHA response and make a case for the validity of the test in avian research, we use the PHA test as a measure of immune response; we refer to the response as the PHA response rather than the immune response. The PHA test was undertaken at Flinders University on 28 captive birds (15 males, 13 females) at the onset of the breeding season (October 2010), and on a different group of 48 captive birds (25 males, 23 females) during the non-breeding season (April 2011). Each bird was injected in the left wing web with a 0.04-ml sample of a 5-mg solution of PHA in 1 ml physiological saline solution (PBS), equivalent to 0.2 mg PHA. If there is a response to the injected PHA, the skin will swell locally. We measured this swelling with a pressure sensitive spessimeter (accurate to 0.01 mm; Mitotoyo, Kawasaki, Kanagawa, Japan) before the injection and 24 h later, using the average of three measurements in each case. The difference between the initial and final wing web thickness was used as an index of the PHA response (Smits et al. 1999). We counted spot number and measured bill and feather colouration 1 day after the PHA test during both breeding and non-breeding seasons, and used the respective spot number and colours indices in the correlation analysis. To test for an association between plumage or bill colouration and the PHA response, we entered the different colour indices (chroma, red-chroma, UV-chroma, hue and brightness) into a principal components analysis to identify the indices that best explained the variation in each sex and season separately.

Body condition and feather lice in the field

If colour traits are honest signals of individual quality, then we predict a correlation between spot number and fitness parameters such as body condition and the prevalence of ectoparasites. Such differences in individual quality are likely to be more pronounced in wild finches because, unlike the captive birds, they are not supplied with ad libitum food and water, and they are more likely to encounter ectoparasites. Between 2004 and 2006, 28 wild individuals (12 females and 16 males) were sampled at Sandy Creek Conservation Park. We took standard morphological measurements (bill length feather, flattened wing length, tail length, tarsus length, mass), manually counted the flank spot number and recorded the presence or absence of ectoparasitic feather lice.

Ethical note

All procedures followed the Guidelines for the Use of Animals in Research (Flinders University), the legal requirements of Australia, and were approved by the Animal Welfare Committee of Flinders University (permit E235). The PHA injections were given 48 h after the birds were moved from the common aviaries to single cages to limit possible stress caused by capture; it has been shown that stress hormones can lead to suppressed PHA response (Ewenson et al. 2003). The PHA test did not have any measurable adverse effects on the birds, as they fed within 30 min after treatment. Birds remained in single aviaries for 24 h after the PHA treatment to reduce possible stress from social interactions in the common aviary.

Statistics

SPSS 18.0 for Windows was used for statistical tests with summary statistics presented as means ± SE. We used MANOVA to compare morphological variables in relation to sex and to compare colour indices from bill, spots and rump feathers between males and females. We used forward stepwise multiple regression analyses to separately test the relationship between the variation in PHA response (dependent variable) and the traits showing sexual dimorphism (tail length, lore width, spot number) and the relationship between PHA response and colour variables for each season. We took three measurements per trait; we have previously shown high repeatability of both colour measurements and the PHA response per bird (Stirnemann et al. 2009).

Results

Morphological measurements

We compared all morphology variables between the sexes using MANOVA, and found significant differences ($F_{9,126} = 3.569$, $P < 0.001$; Wilks' Lamba = 0.745; partial eta square = 0.25). Specifically, males had longer tails and wider lores than females (Table 1).

Bill and plumage colouration

We compared all colour indices between the sexes using MANOVA, and found significant differences ($F_{13,44} = 6.114$, $P < 0.001$; Wilks' Lamba = 0.356; partial eta square = 0.64). Males had higher values of bill hue, bill chroma, rump hue, rump chroma, and rump red-chroma; only the bill spectrum was bimodal, with peaks in the UV (300–400 nm) and red (575–700 nm). Flank spot feathers showed similar spectra between the sexes (Table 2). Also, spot number did not differ significantly between the right and left flank in either sex (Table 2).

Spot number

Spot number differed significantly between the sexes (Table 1). Females had more spots than males, but there was overlap between the sexes (Fig. 1). We tested for a correlation between spot number and body condition (the residuals of mass against tarsus length) and found no significant correlation in either sex (Pearson correlation: males, $r = -0.01$, $P = 0.957$, $n = 68$; females, $r = 0.23$, $P = 0.060$, $n = 67$). There was no significant difference in spot number between captive or wild populations. To test this, we compared the total spot number between the combined population of captive finches held at Adelaide Zoo and Flinders University and the combined wild populations at Monarto Zoological Park and Sandy Creek Conservation Park (ANOVA: $F_{1,159} = 0.17$, $P = 0.678$).

Spot number did not change significantly across years (2009 and 2010) in males (paired t test: Year 1: 56.68 ± 2.53; Year 2: 59.59 ± 2.034; $t_{21} = -1.97$, $P = 0.061$) or females (paired t test: Year 1: 63.21 ± 2.67; Year 2: 65.29 ± 1.94; $t_{13} = -0.75$, $P = 0.47$). For a subsample of 15 individuals, we could test the difference in spot number over three consecutive years (2009, 2010, and 2011) and again, found no significant difference in males ($n = 9$) or females ($n = 6$) (Friedman Test: males, $\chi^2 = 0.76$, $P = 0.682$; females, $\chi^2 = 3.00$, $P = 0.220$). There was no significant difference in the residuals of the regression between spot number in Year 1 and Year 2 for either sex ($P > 0.6$).

Table 1 Morphology and spot number in male and female Diamond Firetails (*Stagonopleura guttata*)

Trait	Male		Female		*F*	*df*	*P*
	Range	Mean ± SE	Range	Mean ± SE			
Bill length	8.7–13.9	11.15 ± 0.10	8.7–12.8	10.92 ± 0.09	1.02	133	0.314
Wing length	61–71	66.23 ± 1.16	61–70	65.9 ± 1.83	1.62	133	0.205
Tail length	30–43	39.61 ± 0.23	34–46	38.72 ± 0.23	5.17	133	**0.025**
Tarsus	15.4–19.0	17.17 ± 0.73	13.9–19.8	16.88 ± 0.09	3.50	133	0.063
Mass	14–28	18.33 ± 0.20	16–24	18.39 ± 0.21	0.003	133	0.955
Breast band	7–23	13.31 ± 0.39	6.7–20.2	12.78 ± 0.40	0.25	133	0.618
Lore width	3.75–5.90	4.82 ± 0.47	3.65–5.63	4.61 ± 0.05	9.26	133	**0.003**
N. Spot (left)	16–44	27.82 ± 0.74	17–44	32.36 ± 0.69	16.25	133	**<0.001**
N. Spot (right)	11–51	27.90 ± 0.77	18–45	32.68 ± 0.66	21.25	133	**<0.001**
N. Spot (total)	33–93	55.63 ± 1.40	36–83	65.04 ± 1.26	21.48	133	**<0.001**

Statistical results are shown for a MANOVA test (male, $n = 66$; female, $n = 69$), with P values <0.05 shown in bold

Table 2 Bill and feather colouration in male and female captive Diamond Firetails

Trait	Male		Female		*F*	*df*	*P*
	Range	Mean ± SE	Range	Mean ± SE			
Bill hue	620.46–648.52	634.50 ± 1.33	594.39–641.54	620.38 ± 2.05	34.34	56	**<0.001**
Bill brighteness	10.05–31.47	19.77 ± 0.95	11.70–27.07	18.32 ± 0.73	0.75	56	0.391
Bill chroma	0.66–1.85	1.10 ± 0.05	0.49–1.46	0.92 ± 0.04	6.32	56	**0.015**
Bill UV-chroma	0.17–0.23	0.21 ± 0.03	0.19–0.24	0.21 ± 0.02	0.51	56	0.480
Bill red-chroma	0.37–0.49	0.42 ± 0.01	0.38–0.48	0.42 ± 0.01	0.004	56	0.950
Spot hue	326.45–344.77	334.37 ± 0.88	325.18–347.98	336.16 ± 1.05	1.46	56	0.231
Spot brightness	28.29–46.81	36.83 ± 0.92	27.012–43.99	34.61 ± 0.78	3.51	56	0.066
Spot chroma	0.48–0.95	0.75 ± 0.02	0.51–1.01	0.75 ± 0.07	0.09	56	0.763
Spot UV-chroma	0.16–0.22	0.19 ± 0.01	0.16–0.21	0.19 ± 0.01	0.18	56	0.671
Rump hue	606.69–618.55	611.38 ± 0.54	595.55–617.60	607.08 ± 1.01	8.89	56	**0.004**
Rump chroma	2.93–4.14	3.72 ± 0.05	2.35–3.94	3.44 ± 0.07	10.02	56	**0.003**
Rump red-chroma	0.75–0.93	0.88 ± 0.01	0.69–0.093	0.85 ± 0.01	7.36	56	**0.009**

Statistical results are shown for a MANOVA test (male, $n = 30$; female, $n = 28$), with P values <0.05 shown in bold

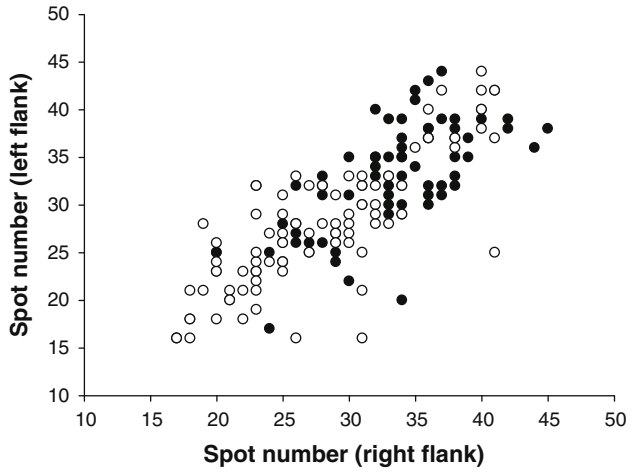

Fig. 1 Spot number on the *right* and *left flanks* of male (*open circles*) and female (*black circles*) Diamond Firetails (*Stagonopleura guttata*)

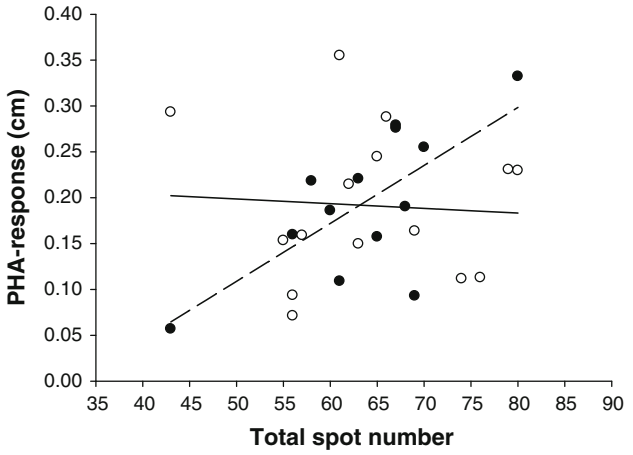

Fig. 2 Relationship between PHA response and total spot number in males (*open circles*, *solid regression line*) and females (*black circles*, *dashed regression line*) during the breeding season. There was a significant relationship for females ($r = 0.69$, $n = 13$, $P = 0.009$) but not males ($r = -0.06$, $n = 15$, $P = 0.821$)

PHA response in captivity

For experimental birds used in the PHA test, there was no significant difference in spot number between the breeding and non-breeding season (ANOVA: season: $F_{1,72} = 0.003$, $P = 0.96$; sex × season: $F_{1,72} = 3.47$, $P = 0.067$). However, as expected, spot number was significantly different between the sexes for the two experimental groups, with females having more spots (sex: $F_{1,72} = 4.97, P = 0.029$). The PHA response was comparable between males and females, and there was no significant effect of season (ANOVA: sex: $F_{1,72} = 0.16$, $P = 0.69$; season: $F_{1,72} = 0.03$, $P = 0.863$; sex × season: $F_{1,72} = 0.035, P = 0.851$).

We compared PHA response in relation to tail length, lore width, and spot number (dimorphic traits) during the breeding and non-breeding seasons. During the breeding season, PHA response in females was predicted by spot number ($F_{1,12} = 10.01$, $r = 0.69$, $P = 0.009$), but not by tail length ($r = -0.30$, $P = 0.336$) or lore width ($r = 0.20$, $P = 0.539$). In males, PHA response was not related to tail length, lore width or spot number during either the breeding season or the non-breeding season (Fig. 2). During the non-breeding season, PHA response varied with spot number in females ($F_{1,22} = 6.52$, $r = 0.49$, $P = 0.018$; but not tail length: $r = -0.11$, $P = 0.617$; or lore width: $r = -0.31$, $P = 0.166$) (Fig. 3).

We used forward stepwise multiple regression to test PHA response in relation to the PCA-derived colour variables (factor loadings and cumulative variance shown in Tables 3 and 4) that were sampled from the bill, flank, spots

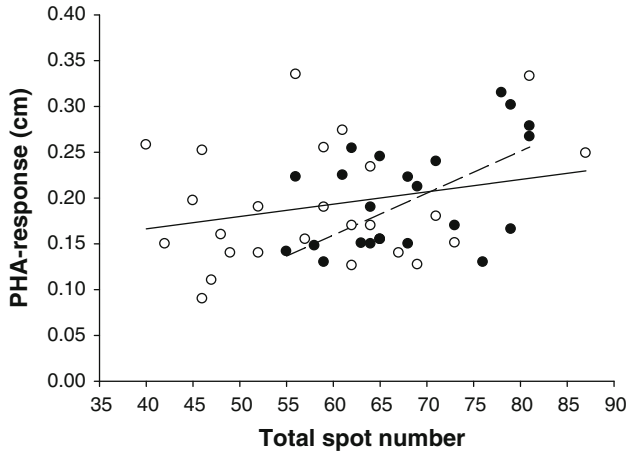

Fig. 3 Relationship between PHA response and total spot number in males (*open circles*, *solid regression line*) and females (*black circles*, *dashed regression line*) during non-breeding season. There was a significant relationship for females ($r = 0.49$, $n = 23$, $P = 0.018$) but not males ($r = 0.24$, $n = 25$. $P = 0.237$)

and rump. During the breeding season, male PHA response was positively correlated with PC1 scores for rump colour ($F_{1,13} = 6.60$, $r = 0.58$, $P = 0.023$) (Fig. 4) and female PHA response was negatively correlated with PC1 scores for spot colour ($F_{1,10} = 6.97$, $r = -0.56$, $P = 0.025$) (Fig. 5). That is, males with a redder rump (higher hue and red-chroma values) had a higher PHA response, and females with higher UV reflectance of the white spots and lower values of hue and chroma had a higher PHA response. During the non-breeding season, there was no effect of any colour variable on PHA response in males ($F_{4,8} = 0.33$, $P = 0.849$) or females ($F_{4,6} = 0.76$, $P = 0.584$).

Body condition and feather lice in the field

Body condition (calculated as the residual of mass against tarsus) was significantly related to total spot number in females (Pearson correlation, $r = 0.887$, $P < 0.001$, $n = 9$), but not males (Pearson correlation, $r = 0.368$, $P = 0.215$, $n = 12$) (Fig. 6). Females with more white flank spots had higher body condition. Birds were commonly infested with feather lice; there was no significant difference in the prevalence of feather lice in females (4 infested, 8 uninfested; 33 %) and males (9 infested, 7 uninfested; 56 %) (Friedman Test: $\chi^2 = 0.317$, $P = 0.576$). There was also no significant relationship between feather lice infestation and the total spot number (independent t test: males, $t = -0.51$, $P = 0.622$: females, $t = 2.08$, $P = 0.071$), or body condition (independent t test: males, $t = -0.50$, $P = 0.631$: females, $t = 0.87$, $P = 0.407$).

Discussion

Our principal findings were: (1) evidence of sexual dimorphism in the apparently monomorphic Diamond Firetail; (2) in captive females, PHA response was correlated with spot number and spot colour; in captive males, PHA response was correlated with rump colour; and (3) in a wild population, female spot number was positively associated with body condition. Some of the sex differences in morphological and colour traits fit the standard expectation for species with choosy females: namely, males are more ornamented in traits that could signal quality and that could be used for female mate choice (Andersson 1994; but see Watson and Simmons 2010). In this study, males had longer tails and wider lores than females, and on average, males also had higher colour reflectance (bill, rump, flank spots) and higher R_{50} reflectance and chroma (bill, rump). We also found that bills reflect UV during the breeding season (there was no sex difference). Notably, the sex differences in bill colour reflectance and bill chroma corroborate the assessment of aviculturists in their attempts to sex Diamond Firetails by ocular estimation (O'Gorman 1980; Higgins et al. 2006).

Table 3 Unrotated factor loadings (component matrix) from principal components analysis (PCA) of the different colour indices of the bill, flank spots and rump in male ($n = 15$) and female ($n = 13$) Diamond Firetails during the breeding season

	Males						Females					
	Bill		Flank spots		Rump		Bill		Flank spots		Rump	
	PC1 61 %	PC2 25.2 %	PC1 67.8 %	PC2 29 %	PC1 70.6 %	PC2 25.3 %	PC1 61.4 %	PC2 22.7 %	PC1 58.5 %	PC2	PC1 71 %	PC2 26.1 %
Chroma	0.94		0.90	0.39	0.99		0.96		0.89		0.98	
UV-chroma	−0.78	0.35	−0.97		n.a.	n.a.	−0.87		−0.98		n.a.	n.a.
Red-chroma	0.95		n.a.	n.a.	0.97		0.90		n.a.	n.a.	0.91	0.37
Hue		0.95	0.92		0.93		0.76		0.71		0.95	
Brightness	−0.78	0.37	−0.32	0.93		0.98		0.98				0.91

Percentage values indicate cumulative variance for each component. Loadings below 0.20 are not reported

n.a. not available

Table 4 Unrotated factor loadings (component matrix) from the principal components analysis (PCA) of the different colour indices of the bill, flank spots and rump in male ($n = 13$) and female ($n = 12$) Diamond Firetails during the non-breeding season

	Males						Females					
	Bill		Flank spots		Rump		Bill		Flank spots		Rump	
	PC1 63.8 %	PC2	PC1 46.9 %	PC2 42.5 %	PC1 74.8 %	PC2	PC1 59.3 %	PC2 20.7 %	PC1 70.4 %	PC2	PC1 81.2 %	PC2
Chroma	0.98		−0.97		0.95		0.90		0.93		0.99	
UV-chroma	−0.86		0.81	0.54	n.a.	n.a.	−0.91		−0.72		n.a.	n.a.
Red-chroma	0.95		n.a.	n.a.	0.81		0.86		n.a.	n.a.	0.97	
Hue				0.91	0.82		0.57	0.62	0.92		0.73	
Brightness	−0.64		0.52	−0.76	0.87		−0.50	0.71	−0.76		0.89	

Percentage values indicate cumulative variance for each component. Loadings below 0.20 are not reported

n.a. not available

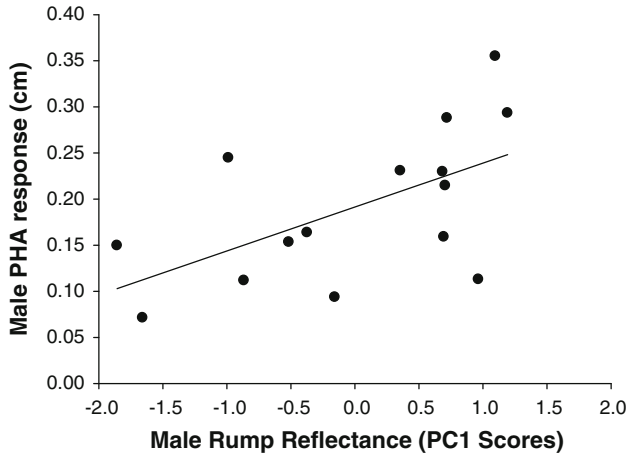

Fig. 4 Relationship between PHA response and PC1 rump reflectance scores in males during the breeding season ($r = 0.58$, $n = 15$, $P = 0.023$)

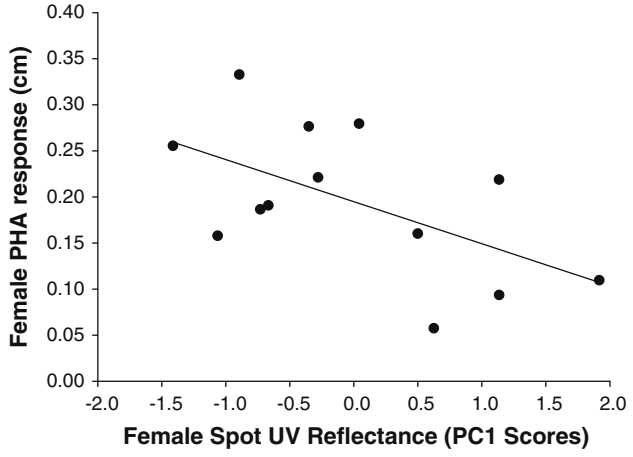

Fig. 5 Relationship between PHA response and PC1 scores (UV reflectance of white flank spots) in females during the breeding season ($r = -0.56$, $n = 13$, $P = 0.027$)

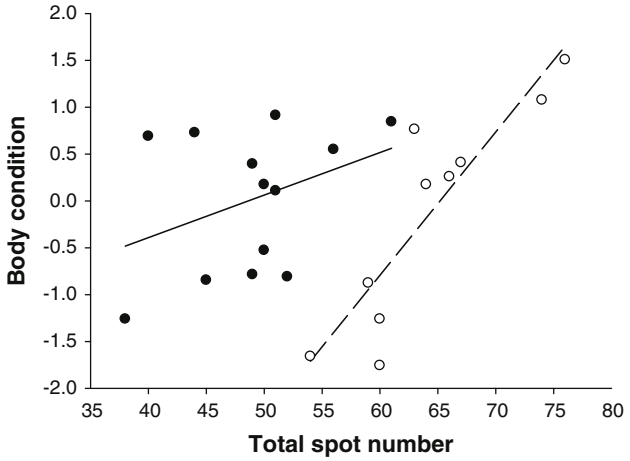

Fig. 6 Relationship between body condition and total spot number in males (*black circles*, *solid regression line*) and females (*open circles*, *dashed regression line*) in a wild population. Body condition was calculated as the residual of mass against tarsus. There was a significant relationship for females ($r = 0.89$, $n = 9$, $P < 0.001$) but not males ($r = -0.37$, $n = 13$, $P = 0.215$)

A previous experimental study—involving carotenoid depletion—showed that the bill colour of Diamond Firetails in the visible range (400–700 nm) was dependant on carotenoids, and males with brighter bills had higher PHA response than duller individuals (Stirnemann et al. 2009). We were unable to repeat this finding in either the breeding or non-breeding season, possibly because birds were not carotenoid depleted; in this study, bill colouration was not related to PHA response. Carotenoids deposited in the bill are defined as mobile carotenoids because they can be easily displaced to activate the immune system and scavenge free-radicals to protect tissues from oxidative damage (Goodwin 1986; McGraw and Ardia 2003; McGraw et al. 2006). Thus, in the 24 h following the injection of PHA, there may have been a rapid reallocation of carotenoids from the bill leading to a decrease in carotenoid-based

colouration (Faivre et al. 2003) and plasma carotenoid levels (Biard et al. 2009). Carotenoids deposited in the feathers are fixed and cannot be mobilised or rapidly changed. We suggest that plumage colouration is a more reliable signal of the immune function. Indeed, we showed that males with redder rumps, a carotenoid-based plumage (McGraw and Schuetz 2004), had a stronger PHA response during the breeding season, which concurs with the findings in other species that individuals with brighter carotenoid-based plumage usually have a higher PHA response or stronger response to parasite infection (Saks et al. 2003; Blount et al. 2003; Mougeot et al. 2009).

Contradicting the convention of more ornamented males, female Diamond Firetails had more flank spots than males. This finding concurs with previous research showing that female birds are generally more spotted than males (Roulin 2003; Swaddle and Witter 1995). Significantly, we found that spot number did not vary with age, which rejects the explanation that sex-related annual mortality explains the finding of sex differences in spot number. But there were sex differences in PHA response: females with more flank spots had a higher PHA response, whereas males did not. Perhaps spot number is a reliable cue for the individual quality of females, as found in other studies. For example, in Common Starlings (*Sturnus vulgaris*), females with more spots on their breasts during the breeding season had earlier ovarian development and thus laid clutches earlier in the season (Swaddle and Witter 1995). Similarly, female Barn Owls (*Tyto alba*) with more black spots had higher parasite resistance (Roulin et al. 2001) and their offspring had increased humoral immuno-competence (Roulin 2004). Our finding of a significant relationship between spot number and PHA response in captive Diamond Firetails is potentially confounded by the abundant food resources and lack of predators and parasites in captivity. Yet, we found that wild Diamond Firetails also showed a positive and strong correlation between female spot number and body condition, supporting the idea that spot number signals individual quality. Finally, we have previously shown, in experimental feeding trials, that female Diamond Firetails with many spots win food contests in social dominance trials, whereas male spot number was not related to feeding dominance (Crowhurst et al. 2012).

We address the colour characteristics of the white spots to gain more insight into their possible signalling function. Previous research tended to view white spots as structural achromatic plumage that is cheap to produce and maintain (Török et al. 2003; Prum 2006). But we show here that birds with white spots, that had high UV reflectance, low chroma and low hue, had a higher PHA response. This association between white plumage and individual quality has been found in few other species. Female Common Eiders (*Somateria mollissima*) with fewer white feathers did not respond to a diphtheria toxoid immune challenge (Hanssen et al. 2006). Similarly, in the South Polar Skua (*Catharacta maccormicki*), the whiteness of the male wing patch was positively related to the response against tetanus (Hanssen et al. 2009). However, these studies only considered brightness, chroma and hue, while here we also showed that females with a higher UV reflectance during the breeding season had a higher PHA response. To our knowledge, this is the first evidence that white UV-chroma correlates with the PHA response, and therefore possibly individual quality in females.

Perhaps the expression of white spots is under genetic control and is not a condition-dependent trait. A genetic mechanism for black spot number has been shown in Barn Owls (Roulin et al. 1998, 2000). Such a genetic mechanism for the expression of white spots could have pleiotropic effects on immunocompetence and other aspects of condition. If white spot number is genetically determined, then what maintains variation in spot number in the population? Why is there not extreme directional selection for males and females with many spots? One hypothesis to explain the observed variation in spot number (between individuals and sexes) is that birds with low or high spot number, for example, pursue different behavioural strategies during foraging or social contexts, and that a mix of strategies confers higher individual fitness payoffs (Roulin 2004; Mckinnon and Pierotti 2010). Our measurements showed no difference across years in adult birds. But spot number could nonetheless be a condition-dependent trait if the variation results from stress during growth and development—which needs to be experimentally tested between the nestling to yearling phase. Finally, it is also possible that spot number is highly heritable whereas UV-reflectance is strongly condition-dependent (Badayaev et al. 2001; Johnsen et al. 2003). These ideas also require future testing.

In conclusion, we have shown that both the number and reflectance of white flank spots correlate positively with female PHA response during the breeding season. Both cues could be signals of female quality. Further research should test the "dual utility hypothesis" of white spots as armament and ornament (Berglund et al. 1996; Griggio et al. 2007). White flank spots can also be used in a multiple signal system (Johnstone 1996; Badayaev et al. 2001), where number (size) and reflectance could indicate different aspects of female quality. Variation in female white spots could function in male mate choice as well as in intrasexual feeding contests and social dominance (Andersson et al. 2002; Doucet and Montgomerie 2003; Wilson et al. 2010; Crowhurst et al. 2012). Finally, the findings add support for a growing body of evidence that white plumage ornaments can be a reliable cue of individual quality (Morales et al. 2007; Hanssen et al. 2009; Lehikoinen et al. 2010).

Acknowledgments We thank the Australian Research Council, Adelaide Zoological Society, South Australian Department for Environment and Heritage, and Conservation Council of South Australia for funding. We thank the Adelaide Zoo for allowing us to measure their captive birds. We thank David Paton for stimulating discussion and assistance with observing and banding Diamond Firetails at Monarto Zoological Park. We thank Wesley M. Hochachka and two anonymous referees for their constructive and helpful comments on a previous version of the manuscript. All procedures complied with the current laws of the country in which they were performed and followed the Guidelines for the Use of Animals in Research (Flinders University) and were approved by the Animal Welfare Committee of Flinders University (permit E190 and E281).

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