FRONTISPIECE. Australasian Gannets are the rarest of the three congeneric gannet species; females and males are similar size but differ in plumage coloration, including the variable black-and-white tail feathers (see Ismar et al. 2014 this issue). Original painting ‘‘Australasian Gannets’’ by Karen Neal; reproduced by permission of the owner (Mark Hauber).

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SEXUAL PLUMAGE DICHROMATISM IN A SIZE MONOMORPHIC SEABIRD

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ABSTRACT.—Data on the extent to which the sexes may differ in their phenotypes are critical for a full understanding of the biology and management of any species. We previously quantified behavioral differences and vocal similarities between genetically-sexed Australasian Gannets (*Morus serrator*). Here, we quantify size monomorphism and plumage dichromatism in this socially monogamous, colonial seabird. In comparison with other sulids, the Australasian Gannet is characterized by low sexual dimorphism indices in various size metrics, and most physical dimensions are statistically similar between adult female and male gannets. In contrast, we found indications of sexually dichromatic plumage traits in the melanin-based, rusty head plumage and in the black-and-white tail feathers. To our knowledge, these findings constitute the first evidence of melanin-generated sexual plumage dichromatism in a size monomorphic seabird species. Using opsin-sequencing, we also confirm that the Australasian Gannet is a visually violet-sensitive species, for which the detection of both gross differences in feather reflectance, and long-wavelength based plumage dichromatism, should be perceptually feasible. However, because of the extensive overlap between females and males in the size and chromatic traits detected here, and in the behavioral and vocal displays reported in previous studies, we advocate for the use of genetic techniques for sex identification in this gannet species. *Received 2 December 2013. Accepted 3 May 2014.*

Key words: gannet, melanin, *Morus serrator*, opsin, sexual selection.

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Sexual dichromatism of the plumage and integuments of birds is often generated by carotenoid pigmentation (e.g., reds, oranges, yellows; Gray 1996, Mays et al. 2004) or structural coloration (e.g., iridescence, ultraviolet; Cuthill et al. 1999, Shawkey et al. 2005). These are, however, rare colors compared to the broad spectrum of melanin-based coloration of feathers and skin, which also constitutes the most common source of coloration across vertebrates and animals in general (McGraw et al. 2005, McGraw 2006, Hubbard et al. 2010).

Most studies of avian sexual dichromatism have focused on songbirds (Irwin 1994, Gray 1996, Mays et al. 2004, Hofmann et al. 2008), which comprise over half of all bird species but fall within only one of the 29 avian orders, the Passeriformes. By comparison, seabirds spanning six orders also exhibit striking feather and integument colors, though these species' sex differences in plumage coloration tend to be less apparent to human observers, and have often required more sensitive spectral analyses (Owens and Hartley 1998, Cuervo et al. 2009, McGraw et al. 2009). Much of the focus on seabird coloration has been on carotenoid- or pterin-based colors, which typically produce oranges and yellows and are thought to function in sexual signaling (Dearborn and Ryan 2002, Velando et al. 2006, Jouventin et al. 2007). In turn, research on melanism in seabirds has primarily focused on intraspecific patterns of plumage polymorphisms (Bretagnolle 1993, del Hoyo et al. 1996).

The Australasian Gannet *Morus serrator* (Order Suliformes) is a colonially breeding, predominantly monogamous seabird (Nelson 1978, Ismar et al. 2010a), in which the sexes are historically reported as similar in plumage coloration (Nelson 1978) and size monomorphic (Daniel et al. 2007). We recently established that this species does not display consistent sex differences in vocalizations (Krull et al. 2012), unlike several closely related booby species (e.g., *Sula* spp.) from the same seabird family (Nelson 1978). Finally, several behavioral traits, including copulation position, but not seaweed carrying, were also found to be poor predictors (~50–80% accuracy) of the genetic sex of individuals (Daniel et al. 2007, Matthews et al. 2008). Thus, accurate and reliable sex identification is only possible by collecting DNA samples from Australasian Gannets (Daniel et al. 2007). Analyses of plumage mono- or dichromatism of known-sex individuals are limited to a handful of studies in this and the other two congeneric species (e.g., Ropert-Coudert et al. 2005), but if found, may become valuable for non-intrusive sex identification, and the assessment of sex-specific behaviors in the field (Matthews et al. 2008, Machovsky Capuska et al. 2014), for both basic biological research and for conservation and management (Zuk and Bailey 2008).

Here, we conducted the largest scale study of size measurements and body weights of genetically sexed, live, breeding adult Australasian Gannets. We used molecular sexing to identify males and females, and employed reflectance spectrometry to characterize the color properties and degree of sexual dichromatism in the rusty head plumage (Fig. 1a); we also characterized the human-visible variation in tail feathers (Fig. 1b) of this predominantly black-and-white seabird. We conducted a genetic analysis of one of the avian eye's short wave-length opsin receptors (SWS1, adapted from Ödeen and Håstad 2003) to independently confirm the report of violet sensitivity in this species (Machovsky Capuska et al. 2011). Finally, we identified the chemical basis of rusty head plumage coloration, because reflectance alone cannot be reliably used to identify the pigmentary and structural basis of plumage diversity (McGraw and Wakamatsu 2004, Griffith et al. 2006).

METHODS

Sample Collection and Sexing.—We studied Australasian Gannets during their breeding season at the Cape Kidnappers gannetry, Hawkes Bay, New Zealand (39° 38′ S, 177° 05′ E, for details for the general methods and study site see Daniel et al. 2007, Ismar et al. 2010a). We drew 50–200 μL of blood from the metatarsal vein in the leg, or the brachial vein in the wing, from each bird handled, stored it in Seutin's lysis buffer, and used a PCR-based genetic method based on the P2/P8 primer set to assign each individual's sex (following Daniel et al. 2007).

Size measurements (as in Ismar et al. 2010b) to the nearest 0.1 mm (Table 1) were taken from 237 different individuals sampled and banded in the 2005/06, 2008/09, and 2009/10 austral springs and summers. Subjects for size measurements were selected when identified as members of pairs observed to display bill fencing (Fig. 1 insert) and to defend a specific nesting site; we did not always succeed in capturing both members of the pair. Weight measurements were taken to the nearest 10 g with a 5 kg Pesola scale, while

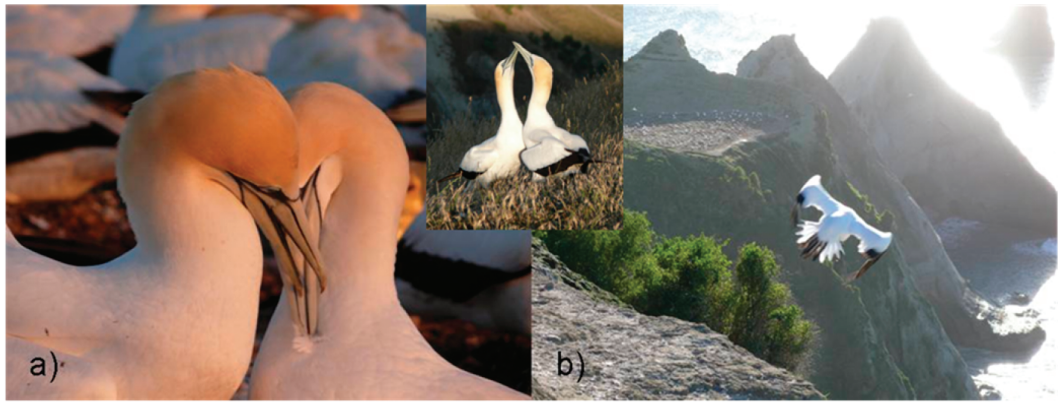

FIG. 1. Australasian Gannets *Morus serrator* at Cape Kidnappers gannetry, New Zealand: (a) head and body plumage coloration of a PCR-sexed male (left) and a female (right), insert: bill-fencing Australasian Gannet pair, (b) most common tail pattern in *Morus serrator*, with the bird showing four outer left white tail feathers, four central black feathers, and four outer right white rectrices. Photo credits S. Ismar.

restraining the birds' wings. Because increased handling time, associated with the additional restraint applied during weighing, likely increases stress (Romero and Romero 2002), only a subsample of 103 birds was weighed for animal ethics reasons, with all weight data collected and analyzed in the October sampling session of the 2005/06 breeding season.

While not an original aim of our field work with gannets (e.g., Matthews et al. 2008, Ismar et al. 2010a, Machovsky Capuska et al. 2012), we also obtained a preliminary set of feather samples from 29 adult Australasian Gannets (17 male, 12 female) captured and DNA sampled, at the gannetry during the early breeding stages of September–October 2007. These individuals were not caught as pairs breeding together, so as to minimize disruptions during the pre-incubation stages of pair-bonding and mate choice at our study site, when the presumably later arriving females had also joined the colony (Ismar et al. 2010a, 2011a). Two rusty colored head feathers from the top of the head, and one white body feather from the back between the wings, were collected from each bird and stored in plastic tubes at 4 °C in the dark until spectral measurements were taken. All handling and sampling occurred under New Zealand Department of Conservation and University of Auckland's animal research ethics permits.

Plumage Color Analyses.—Three reflectance spectra were recorded from the distal part of each

TABLE 1. Morphological measurements and tail pattern score for male and female Australasian Gannets *Morus serrator* sampled from 2005–2009 at Cape Kidnappers gannetry, New Zealand. Sexual Dimorphism Indices (SDI) are calculated according to Lovich and Gibbons (1992), and statistically significant ($\alpha < 0.05$) comparisons are in bold.

	Weight	Culmen length	Bill depth	Bill width	Head width	Tarsus length	Wing chord	Tail length	Tail pattern
	[g]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	score
Male	2,590	89.8	32.1	25.6	48.7	57.4	480	204.2	0.32
SE	248	0.2	0.2	1.7	0.3	0.5	0.8	1.4	0.286 – 0.333
n	54	134	133	134	90	119	131	109	82
Female	2,660	89.1	31.5	25	49.4	58	480	202.8	0.38
SE	223	0.2	0.2	1.5	0.2	0.3	0.8	1.2	0.333 – 0.458
n	47	103	103	102	69	97	101	87	83
t	−1.498	**2.05**	**2.433**	**2.826**	−1.765	−0.973	0.047	0.741	
P	0.137	**0.041**	**0.016**	**0.005**	0.080	0.332	0.967	0.459	**0.01**
(M-F)	−0.10	**0.70**	**0.60**	**0.60**	−0.70	−0.5	0.00	1.40	**−0.06**
SDI [%]	2.64	**0.74**	**1.85**	**2.35**	1.40	0.94	0.01	0.67	**18.75**

TABLE 2. Average reflectance properties and color parameters of head and body feathers from 17 male and 12 female Australasian Gannets *Morus serrator* sampled during the early breeding season at Cape Kidnappers gannetry, New Zealand. Significant differences ($\alpha < 0.05$) are in bold.

Sample	Brightness	UV	Blue	Green	Yellow	Red	Hue	Chroma
				White body plumage				
Male	34,789	0.215	0.198	0.196	0.194	**0.195**	658.540	0.001
SE	1,090	0.004	0.001	0.002	0.002	**0.001**	22.600	0.000
Female	40,272	0.201	0.199	0.200	0.198	**0.201**	658.620	0.001
SE	3,263	0.007	0.001	0.002	0.002	**0.002**	25.940	0.000
t	−1.760	1.824	−0.715	−1.136	−1.272	**−2.802**	−0.002	−1.519
P	0.090	0.080	0.481	0.267	0.215	**0.038**	0.998	0.141
				Rusty head plumage				
Male	9,357	0.093	0.157	0.218	0.247	0.284	698.81	**0.002**
SE	726	0.003	0.002	0.002	0.002	0.003	0.150	**0.000**
Female	9,635	0.103	0.161	0.218	0.245	0.273	698.390	**0.002**
SE	924	0.006	0.004	0.001	0.004	0.006	0.340	**0.000**
t	−0.240	−1.553	−0.961	0.212	0.489	1.972	1.275	**2.265**
P	0.810	0.132	0.345	0.833	0.628	0.059	0.213	**0.031**

collected feather using an OceanOptics HR 2000 high-resolution spectrometer, illuminated by a DT mini-lamp and using OOIBase32TM operating 136 software (Ocean Optics, Inc. Dunedin, FL, USA). Each fine rusty head feather was bundled around the rachis using the narrow end of a 200 μL pipette tip, to provide a representative feather surface. The recorded spectra consisted of three replicates over a 1500 ms integration time from the same spot of the feather, which were automatically averaged by the software. Illumination arrangement and spectrophotometer settings followed Igic et al. (2010) and all measurements, including standard reflectance calibrations, were taken as in Ismar et al. (2011b). Chromatic and achromatic plumage parameters were calculated from each spectrum as in Endler (1990) (Table 2). Because of the fine filamentous structure of the rusty feathers, the measurement with the highest brightness score was taken for each head feather for the analyses of sexual dichromatism. By contrast, three spectra were obtained and averaged for every white body feather.

To quantify variation in black-and-white tail coloration, the human-assessed patterning of each tail feather (Fig. 1b) was estimated using a three-point scale as follows: completely black = 1, variegated (feathers showing both black and white coloration) = 0.5, and white = 0. We then divided the sum of all scores by the total number of tail feathers. We recorded data only for the tail patterns of captured birds that displayed a complete set of 12 rectrices (Fig. 1b), and these were compiled for 73 birds in 2005/2006 and 92 birds across the breeding seasons 2007–2010 (including a total of 83 female, 82 male gannets). To test for age differences on tail coloration, regression analysis was performed for each sex separately, with minimum age as the independent variable and proportion of black rectrices as the dependent variable. Because most birds at our colony were first captured and banded as adults, their age was assigned a minimum value of 4 years at the time of banding (Nelson 1978), plus the time in years elapsed since the birds were first banded.

Genetic Analyses of SWS1 Opsin.—We extracted genomic DNA from blood samples of 1 male and 1 female Australasian Gannet by using the phenol-chloroform protocol (Sambrook and Russell 2001). To assess ultraviolet (UVS) or violet (VS) sensitivity (Aidala et al. 2012a,b), primers SU149a and SU306b were used to amplify a 119 bp region of the UVS/VS opsin gene (SWS1), known to encode a critical 'spectral tuning' region (Ödeen and Håstad 2003). Polymerase chain reactions (PCRs) were conducted in 20.0 μL final reaction volumes (comprising 0.2 mM of each dNTP, 0.4 μM of each primer, 2.5 mM $MgCl_2$, 1 × Applied Biosystems GeneAmp Gold buffer, 0.5 units of Applied Biosystems AmpliTaq Gold polymerase, and ~20 ng of genomic DNA). Cycle conditions began with a 7-min denaturing step at 95 °C, followed by 37 cycles of 95 °C for 30 secs, 52 °C for 30 secs, and 72 °C for 30 secs,

with a final extension step of 72 °C for 10 mins. The PCR products were purified from 2% agarose gels using GE Healthcare Illustra GFX columns. Sequencing was conducted using Applied Biosystems Big Dye v3.1 chemistry and electrophoresis on an ABI 3130XL genetic analyzer. Sequences were edited and aligned with Sequencher for Macintosh (Gene Codes, Ann Arbor, MI), and aligned with avian SWS1 partial or complete coding sequences available on GenBank.

Feather Pigment Analyses.—Carotenoid and melanin analyses of the rusty feathers followed the methods detailed in McGraw and Wakamatsu (2004). The tips of these head feathers were stored in a sealed dark container until pigment analysis. Feathers were subjected to acidified-pyridine extraction followed by hexane:*tert*-butyl methyl ether and quantified using a Waters™ 717plus Autosampler high-performance liquid chromatograph (HPLC, Millipore Corp., Bedford, MA), fitted with a Develosil RPAqueous RP-30 column (250 × 4.6 mm, Nomura Chemical Co. Ltd., Aichi, Japan), and with the column temperature set at 27 °C; as positive controls for the extraction method we used yellow, carotenoid-pigmented contour feathers of the American Goldfinch *Spinus tristis* (following McGraw et al. 2001).

Regarding melanin quantification, to determine eumelanin concentrations in a separate set of rusty head feathers, we extracted pigments using H_2SO_4, oxidized with 3% $KMnO_4$, and followed it with HPLC, whereas phaeomelanin concentrations were determined using 57% hydriodic acid at 130 °C in the presence of H_3PO_2, and followed with HPLC; we used commercially sourced pyrrole-2,3,5-tricarboxylic acid (PTCA) and 4-amino-3-hydroxyphenylalanine (4-AHP) standards as controls, respectively (following McGraw and Wakamatsu 2004). These compounds were synthesized according to d'Ischia et al. (2013).

Statistical Analyses.—Morphometric data were first tested for differences between the sexes using two-tailed unpaired *t*-tests, with $\alpha = 0.05$. To assess whether morphological parameters were different between the sexes, generalized linear mixed models (GLMM) were run in JMP®7 (SAS Institute Inc. 2007), with culmen length (exposed dorsal ridge of the upper mandible of the avian bill), bill depth and width, head length, tarsus length, tail length, and wing chord as continuous response variables, and sex as a nominal predictor variable. A size dimorphism index (SDI) from the male relative to the female mean values of all collected measurement types was calculated, as outlined by Lovich and Gibbons (1992):

$$SDI = -(\bar{x}_{male}/\bar{x}_{female}) + 1, \text{ if males were larger,}$$
$$SDI = (\bar{x}_{female}/\bar{x}_{male}) - 1, \text{ if females were larger.}$$

The extent of sexual size dimorphism (SSD) [%] reported was subsequently calculated as $SSD = |SDI| * 100$.

We also used two-tailed unpaired *t*-tests to assess sex differences in physical color parameters for both rusty and white feathers. We then again employed GLMMs on plumage dichromatism indices, with sex as a binomial predictor variable. To account for considerable intra-sexual variation, both intra- and inter-individual, that may mask or outweigh inter-sexual differences in phenotypes (e.g., vocalizations; Krull et al. 2012; also see Sommerfeld et al. 2013 for behavioral traits of the related Masked Booby *Sula dactylatra*), we explored statistical models of sex differences where individual identity was nested within sample identity, and report those with significant predictor terms below.

Tail coloration data of males and females were tested for differences by applying two-sample *t*-tests and Kolmogorov-Smirnov tests at a significance level of $\alpha = 0.05$ and a discrimination threshold $d_{max} = 1.36/\sqrt{165} = 0.106$.

RESULTS

Sexual Size Monomorphism.—Body mass, which is commonly used to establish the extent of dimorphism in seabirds (Fairbairn and Shine 1993), wing chord, tarsus length and tail length did not differ significantly between the sexes in *M. serrator* (Table 1). This was despite our large sample size (100+ individuals per sex) and small measurement error for each trait. By contrast, exposed culmen length, bill width, and bill depth were significantly greater in males than in females, but the sexes differed only by 0.7–2.5% for these traits; several of these differences were at the sub-millimeter scale in absolute values (Table 1).

Consistent with these findings, the suite of morphometric parameters measured did not predict sex (overall fit: $\chi^2 = 7.223$, $P = 0.51$, $r^2 = 0.055$, df = 229, $F = 8$). By contrast, bill dimensions alone (culmen length, bill width, and bill depth) significantly predicted sex, albeit with a low predictive power (overall fit: $\chi^2 = 44.72$, P

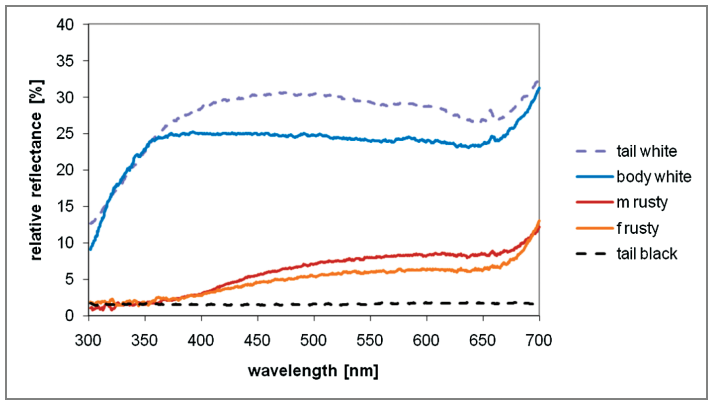

FIG. 2. Reflectance spectra of Australasian Gannet plumage components, showing representative examples taken from different feather types collected from females (f) and males (m) and measured in the laboratory. The vertical order of the legends of feather types (from ''tail white'' through ''body white,'' and ''m rusty'' and ''f rusty'' to ''tail black'') matches the vertical order of the reflectance curves at the wavelength of 500 nm.

< 0.001, $r^2 = 0.087$, df = 233, $F = 9$); both bill depth ($\chi^2 = 4.35$, $P = 0.036$) and bill width ($\chi^2 = 9.47$, $P = 0.002$) contributed significant parameter estimates as predictors of sex.

Head and Body Plumage.—Relative reflectance spectra and total chroma of the sampled feathers differed significantly between male ($n = 17$) and female ($n = 12$) Australasian Gannets (Table 2); we detected significantly higher total chroma in male compared to female rusty head feathers, as well as an indication of higher red chroma in male than in female rusty feathers (Fig. 2). An effect test in a combined model analysis for sex, with bird identity nested within sample identity, of red chroma in the rusty head plumage showed that only sex was a significant predictor ($\bar{x}_{male} = 28.37$ +/− 0.26%, $\bar{x}_{female} = 27.27$ +/− 0.56%, df = 26, $F = 5.13$, $P = 0.029$). By contrast, white body plumage in males was significantly less chromatic in the long wavelength region than female body plumage (Table 2). There was no significant difference in UV reflectance of white body plumage between males and females. Achromatic reflectance parameters also did not differ between male and female plumage: there were no significant sex differences in total brightness of either rusty head, or white body feathers (Table 2).

Tail Feathers.—There was no significant variation in the proportion of black in the tail plumage across the different years of sampling for either sex (males $t = 0.538$, df = 80, $P = 0.60$; females $t = 1.231$, df = 81, $P = 0.22$). Overall, female gannets ($n = 83$) had a markedly higher proportion of black in their tail feathers than males ($n = 82$) (Table 1). Percentage data of black tail proportion were not normally distributed (Fig. 3), and the non-parametric Mann-Whitney *U*-test showed a significant difference between the sexes ($U = 2{,}595$, $t = 5{,}998$, $P = 0.005$).

There was no statistical correlation between the estimated minimum age of individual birds and proportion of black tail rectrices in the total sample of 165 gannets ($r^2 < 0.001$, $P = 0.65$). Separate regression analyses showed no statistical support for a consistent change in black tail proportions with age within females ($y = -0.0107x + 0.4407$, $r^2 = 0.036$, $P = 0.084$), or within males ($y = 0.006x + 0.287$, $r^2 = 0.028$, $P = 0.13$).

Opsin Sequencing.—We amplified ~119 bp of the SWS1 opsin gene, which encodes a region critical in spectral tuning, from genomic DNA extracted from both a male and a female Australasian Gannet (Genbank accession numbers EU651857 and EU651858). Both sequences yielded a predicted set of protein sequences at the three critical 'spectral tuning' residues to suggest that Australasian Gannets' SWS1 proteins absorb maximally at violet frequencies (Håstad et al. 2005). Specifically, the SWS1 sequences of Australasian Gannets are identical to those recently reported for the VS SWS1 sequence of this species by Machovsky Capuska et al. (2011), and to those of the congeneric Northern Gannet *M. bassanus* (Håstad et al. 2005; GenBank acc. no. Y960721).

Feather Pigment Analyses.—No carotenoids were detected above the instrumentational threshold in our HPLC analyses of lipid extracts from

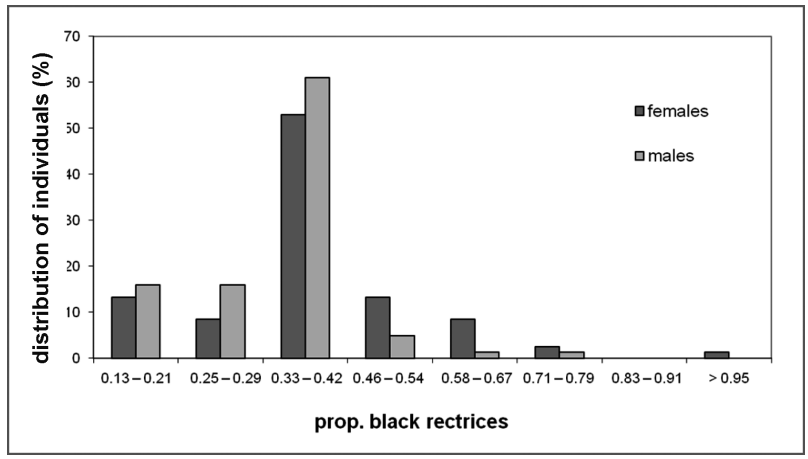

FIG. 3. Tail pattern scores of PCR-sexed male and female Australasian Gannets; distributions of individuals with different proportions of black rectrices in females (dark grey) and males (light grey).

rusty gannet feathers (detection limit = 1 μg pigment per g feather). Instead, low quantities of melanin generate the rusty coloration in head plumage of both male (eumelanin measured as PTCA: 34.9 ng/mg, SD = 10.81 ng/mg, and phaeomelanin measured as 4-AHP: 11.36 ng/mg, SD = 3.57 ng/mg) and female (PTCA 38.62 ng/mg, SD = 12.80 ng/mg, 4-AHP 11.31 ng/mg, SD = 4.53 ng/mg) Australasian Gannets. There was no sex difference in the concentration of extracted pigments (unpaired *t*-tests: PTCA $t = -0.868$, $P = 0.39$, 4-AHP $t = 0.038$, $P = 0.97$). Neither in males nor in female gannets did the proportion of black rectrices in the full set of tail feathers correlate with concentration of head plumage pigments (as 4-AHP/PTCA, male $r^2 = 0.111$, $P = 0.19$, female $r^2 = 0.066$, $P = 0.47$).

DISCUSSION

Seabird taxa that display such slight differences in morphology, at SDI <5% of respective measurements, as detected here for *M. serrator*, are typically treated as functionally monomorphic (Nelson 1978, Lewis et al. 2002). In our data sets, both the statistically significant (SDI range: 0.74–2.35%) and the non-significant (SDI: 0.01–2.64%) sex difference measures yielded small and overlapping ranges of SDI, implying a lack of biologically meaningful dimorphism in any of these individual measures. Our conclusion, therefore, is that Australasian Gannets are sexually size monomorphic.

This conclusion is also supported by the comparison of our data on the sex-specific size similarities for the Australasian Gannet, with the consistently greater dimorphism indices (SDI) reported for other members across the sulid seabird family of gannets and boobies (Table 3). For example, the SDI of weight of Australasian Gannets (3%) was lower than that reported for the congeneric Cape Gannet *M. capensis,* in which females are slightly (4%), but also statistically significantly, heavier than male conspecifics (Bijleveld and Mullers 2009). Even in the least sexually dimorphic of the booby species, the Abbott's Booby *Papasula abbotti,* females are ~7% heavier than males (Nelson 1978). In wing chord and tail length, our measurements of the Australasian Gannet show even less evidence for sexual dimorphism than reported for the Cape Gannet and Northern Gannet, or, generally, for the other sulid species (Table 3).

In contrast to our extensive sampling of size measurements, sexual dichromatism was assessed on a preliminary basis in our study, as it was based on a much smaller sample size of individuals for which no clear assignment of breeding status was made at our study colony. Nonetheless, we detected consistent dichromatism in both the rusty head plumage and in the proportion of black tail pigmentation between the two sexes of Australasian Gannets. This provides the first evidence of sex differences in morphology in this otherwise largely size- and vocally monomorphic species (Krull et al. 2012).

TABLE 3. Sex-specific mean morphological measurements of the Sulidae, Sexual Dimorphism Indices (SDI), calculated as in Lovich and Gibbons (1992).

Species		Weight g	Culmen length mm	Bill depth mm	Bill width mm	Tarsus length mm	Wing chord mm	Tail length mm	Source
Sula sula	Male	891.2	81.8	27	22.5		383.9	251.2	Weimerskirch
	Female	1020.4	84.8	28.7	23.2		394.1	244.3	et al. 2005
	SDI	0.145	0.037	0.063	0.031		0.027	−0.03	
S. sula	Male	840	78.9	12.5		35.5	387		Lormee et al.
	Female	987	81.1	13.1		37	400		2005
	SDI	0.175	0.028	0.048		0.042	0.034		
S. sula	Male	1,035	81				405		Lewis et al.
	Female	1,182	84				417		2005
	SDI	0.142	0.037				0.03		
S. sula	Male	940	86	27.5		38	379	215	Nelson 1978
	Female	1,070	90	28		40	385	205	
	SDI	0.138	0.047	0.018		0.053	0.016	0.05	
S. nebouxii	Male	1,319	99.5			53	409.2		Zavalaga et al.
	Female	1,723	106.8			57.9	432.7		2007
	SDI	0.306	0.073			0.092	0.057		
S. nebouxii	Male	1,280	106	33.5			432		Nelson 1978
	Female	1,800	114	35.3			457		
	SDI	0.406	0.075	0.054			0.058		
S. variegata	Male	1,300	94	30		48	386	164	Nelson 1978
	Female	1,520	99	32		50	429	171	
	SDI	0.169	0.053	0.067		0.042	0.111	0.043	
S. leucogaster	Male	1,077	97				415		Lewis et al.
	Female	1,491	103				427		2005
	SDI	0.384	0.062				0.029		
S. leucogaster	Male	960	96.2	32		44	380	186	Nelson 1978
	Female	1,260	102	34.2		47	396	181	
	SDI	0.313	0.06	0.069		0.068	0.042	0.03	
S. abbotti	Male	1,500	106.1	37.5			449	219	Nelson 1978
	Female	1,600	113.3	39.8			461	231	
	SDI	0.067	0.068	0.061			0.027	0.055	
S. granti	Male		101.9	33.3		54.1	437.8	175.4	Pitman and Jehl
	Female		105	35.4		57.4	460.8	179.9	1998
	SDI		0.03	0.063		0.062	0.053	0.026	

TABLE 3. Continued.

Species		Weight g	Culmen length mm	Bill depth mm	Bill width mm	Tarsus length mm	Wing chord mm	Tail length mm	Source
S. dactylatra	Male		104.3				413	171	O'Brien and Davies
	Female		103.9				425	177	1990
	SDI		0.004				0.029	0.035	
S. dactylatra	Male		108.4	41.9	31.3	63.9	459.4	179.5	Ismar et al.
	Female		113.6	43.7	31.8	66.4	476.3	190.3	2010c
	SDI		0.048	0.043	0.016	0.039	0.037	0.060	
S. dactylatra	Male		108.3				442	187	Pitman and Jehl
	Female		109.9				452	190	1998
	SDI		0.015				0.023	0.016	
S. dactylatra	Male	1,639	106.8	37.3		55	432	188	Nelson 1978
	Female	1,880	109.3	38.1		57	456	192	
	SDI	0.147	0.023	0.021		0.036	0.056	0.021	
Morus capensis	Male	2,526	91.5			60.2	464		Bijleveld and Mullers
	Female	2,620	89.6			60.2	464		2009
	SDI	0.037	0.021			0	0		
M. bassanus	Male		100.1						Lewis et al.
	Female		99.2						2002
	SDI		0.009						
M. bassanus	Male	3,073	100	35			513		Nelson 1978, 2002
	Female	3,110	99.8	34.3			510		
	SDI	0.012	0.002	0.02			0.006		
M. serrator	Male	2589.6	89.8	32.1	25.6	57.4	480	204.2	this study
	Female	2658.1	89.1	31.5	25	58	480	202.8	
	SDI	0.026	0.008	0.019	0.024	0.01	0	0.01	

Even though we found that total melanin pigment concentration in rusty head plumage did not differ between the sexes, the structure and arrangement of the involved melanosomes may cause distinctive reflectance properties (McGraw et al. 2005, Shawkey et al. 2006). In turn, the detected higher proportion of black rectrices in females constitutes a methodologically conservative calculation of sexual dichromatism in this plumage trait, as all variegated feathers were scored as 0.5, irrespective of the proportions of white and black in the feather. Consequently, our metric should be regarded as a rough estimate of the quantitative patterns of sexual dichromatism of tail coloration in the Australasian Gannet. Nevertheless, given our own confirmation of Machovsky Capuska et al. (2011) that the visual system of this gannet species is not tuned for ultraviolet wavelengths, we predict that both types of chromatic differences between the sexes would be perceived when viewed by the gannets' eyes and processed by their visual system. However, in the absence of any experimental manipulations, we cannot speculate about a potential communication or other biological function, if any at all, subserved by sexual plumage dichromatism in the Australasian Gannet.

Overall, the results reported here provide a valuable comparison for our overview of SDI across sulid seabirds (Table 3), and underline the continued need to assess previously undetected patterns of sex dichromatism in other putatively monomorphic species (Eaton 2005). Regarding the practical implications of our data for sexing birds in the hand or by observation in the field, we conservatively argue against the use of morphological or behavioral metrics for the Australasian Gannet. This is because, in this species, size is not sexually dimorphic (Table 1: this study); and vocalizations show more inter-individual than intersexual differences (Krull et al. 2012). Even those aspects of behavior and plumage coloration that do show sexual dichromatism, are not diagnostic because of inter-individual variation (e.g., seaweed carrying: Matthews et al. 2008; Figs. 1–2: red chroma, this study) and extensive overlap between the sexes (Fig. 3: proportion of tail black, this study), rendering field-identification of the sexes unfeasible and genetic methods of sex assignment necessary (Daniel et al. 2007).

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