Pigment and Morphometric Variation in the Buff-rumped Thornbill

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Though the Buff-rumped Thornbill *Acanthiza reguloides* and Varied Thornbill *A. squamata* of the 1926 RAOU Checklist are now combined as a single species (Schodde 1975), the number of subspecies (*sensu* Ford 1974) is uncertain, ranging from four (Mayr & Serventy 1938) to none (Storr 1984). Differences between extreme forms are strong (Table 1, Fig. 1), *reguloides* having a buff-coloured rump and belly and *squamata* a rich yellow rump and belly, but there is no quantitatively based analysis of geographic variation in *A. reguloides* designed to reveal possible zones of secondary contact and intergradation. Accordingly, we undertook this study of the bird's integumentary pigments and morphometrics.

Skins of 138 sexed adults from South Australia, Victoria, New South Wales and Queensland were examined from the American Museum of Natural History (AMNH), Australian Museum (AM), Australian National Wildlife Collection (ANWC), Museum of Victoria (MV), Queensland Museum (QM), South Australian Museum (SAM) and Western Australian Museum (WAM).

Yellow colouration was assessed as follows. Specimens were pooled according to locality. Some belly and rump feathers were removed and treated with methanolic 5% KOH solutions until the feathers appeared devoid of yellow. Filtered extracts were dotted onto thin layer chromatography (TLC) plates consisting of either alumina or silica gel and developed with various solvents to determine the number of carotenoids.

Carotenoids have characteristic visible spectra (Foppen 1971; Davies 1976; Moss & Weedson 1976). Consequently, the spectra of the carotenoid in methanol and light petroleum were recorded between 300 and 600 nm. The carotenoid was transferred from methanol by adding petroleum spirit and deionized water, and gentle shaking. The spectrum of a neutralised methanolic solution was also recorded to eliminate the possibility of the presence of

TABLE 1 Differences in colouration between adults of populations of the Buff-rumped Thornbill *Acanthiza reguloides*.

Character	Subspecies		
	squamata	*nesa*	*reguloides*
Belly	yellow	cream	buff
Throat	yellow	pale yellow	pale buff
Rump	yellow	pale yellow	buff
Back	olive	less olive	olive brown
Tips of tail	pale	pale to buff	buff
Base of tail	pale ochraceous	pale ochraceous	ochraceous
Forehead scallops	pale grey	pale grey	buff

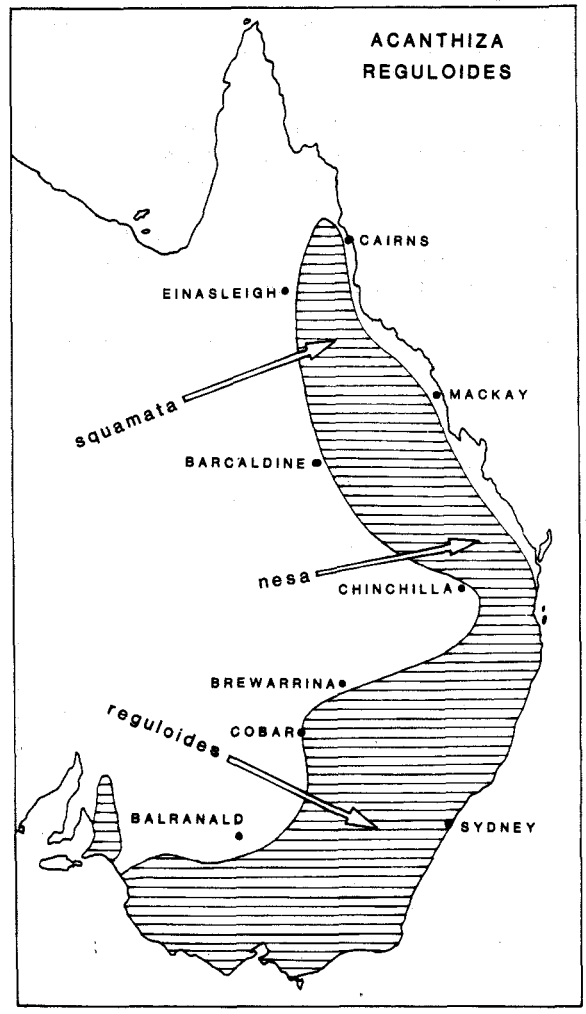

FIGURE 1. Distribution of *Acanthiza reguloides*.

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carboxylic acid groups (Moss & Weedon 1976).

Partition coefficients provide another common technique for identifying carotenoids (Petracek & Zechmeister 1956; Foppen 1971). These were determined by extracting the feathers with 5% KOH in methanol, aqueous 95% methanol and in aqueous 80% methanol, adding equal volumes of light petroleum to the filtered extracts, equilibrating, and measuring the absorbance of each phase at the wavelength of maximum absorbance.

The relative amounts of carotenoid in feathers from 44 localities were determined by weighing the yellow and buff portions of the feathers, extracting with 5% KOH in methanol, filtering, rinsing, and making up to a known volume with the solvent. Relative absorbances at the peak wavelength were obtained by correcting for volume of solvent and weight of feathers by using the formula: relative absorbance = observed absorbance × volume/weight × 100.

Feathers were examined under a low-powered microscope to assess the pigment producing the buff colouration, which was presumed to be phaeomelanin.

The wing, tail, bill (tip of culmen to base of skull) and tarsus were measured as described elsewhere (Ford 1985). Statistical tests utilised the *t*-statistic.

Results and discussion

The TLC plates indicated only one carotenoid. Its absorption spectrum in methanol had peaks at 420, 442 and 472 nm; in neutralised methanol, the same three; and in light petroleum spirit, 420, 444 and 476 nm (Fig. 2). Foppen (1971) listed maximaa of 420, 445 and 475 nm for lutein (3,3'-dihydroxy-α-carotene) in light petroleum. The partition coefficients of the acanthizid carotenoid were 17:83 between aqueous 95% methanol and light petroleum, and 42:58 between aqueous 80% methanol and light petroleum. These compare respectively with 12:88 and 43:57 for lutein between the methanol solutions and hexane (Petracek & Zechmeister 1956; Foppen 1971).

Variation in the amount of yellow carotenoid with latitude revealed a cline, apparently with increased variability in the region of 28°S (Fig. 3). Specimens with buff feathers, as well as those with yellow, contained the yellow carotenoid. The correlation coefficient for all samples was 0.68 (2-tailed, P = 0.0000) and the regression equation was Absorbance = -0.319 Latitude + 17.867. The slope of the regression line was the same with and without samples from South Australia.

Bright yellow specimens only came from northern Queensland south to the Clarke Range and inland to the Burra Range; light yellow specimens only from the lower Dawson and Bunya Range south to the Brisbane area, except for two bright yellow birds out of nine from Emu

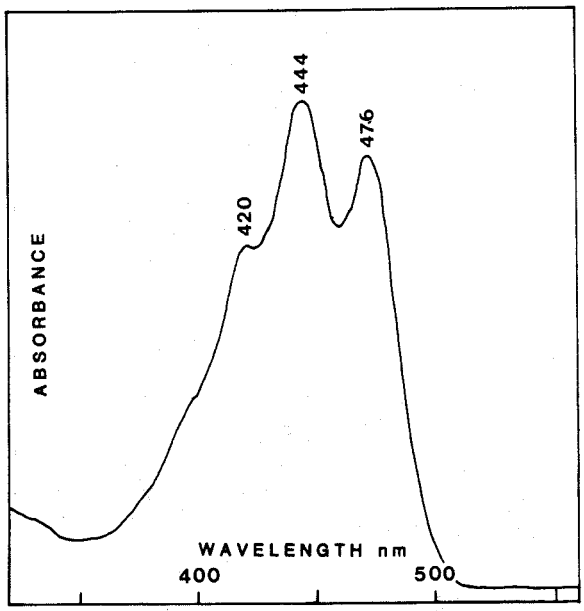

FIGURE 2. Absorption spectrum of carotenoid in light petroleum spirit extracted from feathers of *Acanthiza reguloides*. The triple-peaked spectrum and absorption maxima are characteristic of lutein.

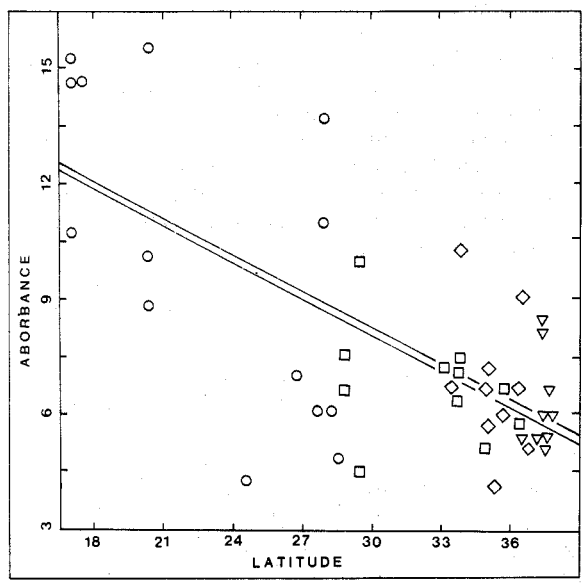

FIGURE 3. Relative absorbance versus latitude for feather samples for various localities. Circles denote localities in Queensland; squares, New South Wales; diamonds, Victoria; and triangles, South Australia. The lower regression line includes South Australian specimens and the upper excludes them.

Vale; buff specimens with an olive dorsal appearance only from north-eastern New South Wales (Tenterfield, Copmanhurst and possibly Emmaville); and typically buff specimens only from south of these localities to the Mt Lofty Range. Under a low-powered microscope, buff birds had brown melanin granules in the distal portion of barbules, and yellow carotenoid in the barbs and basal portion of the barbules; pale yellow birds had dark tipped barbules only on the terminal part of the feather. Buff was produced by this apposition of yellow and brown pigments. The replacement of carotenoid by phaeomelanin in the barbules and an apparently reduced concentration of yellow elsewhere contributed to the lower lutein values in southern birds (Fig. 3).

Morphometric analyses revealed only slight trends (Table 2), so comparisons were expressed in terms of yellow specimens (those north of 28°S) and buff ones (those south of 28°S, *cf.* Ford 1985). Females have shorter wings and tails than males, and similar bills and tarsi. Females but not males of northern populations are slightly shorter in wing and tail than southern ones. Both sexes of northern populations have slightly shorter bills than southern ones.

Though Mayr & Serventy (1938) recognised four subspecies in *A. reguloides* (*squamata, nesa, reguloides* and *australis*), sharp subspecific boundaries are hard to locate. Condon (1968) accepted *australis* for the Mt Lofty Range population, believing its rump was more brownish-yellow and its back darker than in *reguloides* but differences in pigmentation between these forms are meagre (Fig. 3) and the two populations are possibly geographically continuous (Fig. 1; Blakers *et al.* 1984; *pace* Mayr & Serventy 1938), albeit tenuously.

Except for some brightly coloured specimens from Emu Vale (in the Granite Belt of the Great Dividing Range) in a vast area otherwise occupied by pale yellow birds, the boundary between *squamata* and *nesa* would be fairly sharp, coinciding with the dry belt of brigalow country stretching inland from Broad Sound. Interestingly for the Brown Thornbill, Boles (1983) also placed the break between the yellow-bellied, olive-green backed *Acanthiza p. mcgilli* and the duller coloured *A. p. pusilla* at this barrier. Unfortunately, specimens of *A. reguloides* have not been collected at critical localities, e.g. Expedition, Carnarvon, Kroombit Tops, Connors and Drummond Ranges, but H. Nix (pers. comm.) believes these are populated by yellow birds. North of Blackdown Tableland (Expedition Range), Consuelo Tableland (Carnarvon Range) and the Drummond Range, the species generally occurs above 500 m (Storr 1984), so its distribution is probably patchy and perhaps discontinuous (*pace* Blakers *et al.* 1984), similar to several other south-eastern species ranging into north-eastern Queensland. Until more specimens are collected from localities intermediate between the known distribution of *squamata* and *nesa*, no satisfactory conclusion may be made on whether these forms should be accepted as subspecifically distinct.

A sharp boundary between pale yellow birds (*nesa*) and buff ones (*reguloides*) may occur about in the region of the axis of the McPherson Range (latitude 28°S). Specimens immediately south of this are quite buff, like southern ones,

TABLE 2 Comparisons between populations of *Acanthiza reguloides* north and south of the McPherson Range (latitude 28°) and between males and females within populations.

Sex	Character	Northern birds	Southern birds	*t* value	*P* value
Males	WING	52.3 1.36 (50-55)	52.6 1.58 (49-56)	1.15	0.26
Females	(mm)	50.0 1.12 (49-52)	52.1 1.49 (49-56)	4.72	0.0003
t value		4.84 (24,9)	1.86 (64,41)		
P value		0.0001	0.07		
Males	TAIL	41.4 1.21 (39-44)	40.9 1.42 (38-44)	1.73	0.09
Females	(mm)	39.0 1.12 (37-40)	40.0 1.58 (37-44)	2.35	0.032
t value		5.40 (24,9)	2.76 (63,41)		
P value		0.0001	0.007		
Males	CULMEN	11.8 0.38 (10.8-12.3)	12.2 0.38 (11.2-13.2)	4.30	0.0001
Females	(mm)	11.5 0.37 (11.0-12.1)	12.0 0.37 (11.3-12.7)	3.64	0.003
t value		1.59 (23,9)	1.62 (63,37)		
P value		0.13	0.11		
Males	TARSUS	17.3 0.56 (16.0-18.0)	17.8 0.57 (16.5-19.0)	3.54	0.001
Females	(mm)	17.2 0.56 (16.5-18.0)	17.6 0.59 (16.0-19.0)	1.96	0.074
t value		0.76 (24,9)	2.07 (64,41)		
P value		0.46	0.042		

Data for each variable are mean, standard deviation and (range). Numbers of males and females are given in brackets after *t* value between sexes. Tests are two-tailed.

but may have more yellow pigment in the feathers and so approach specimens from just north of the McPherson Range in this respect. The great variability of lutein in specimens from lower south-eastern Queensland (Fig. 3) indicates the possibility of secondary intergradation. Nevertheless, if yellow is decreasing and phaeomelanin increasing clinally from north to south, a population deficient in both pigments (as in *nesa*) would be expected at some geographically intermediate region, and so seemingly sharp boundaries between *squamata* and *nesa* and between *nesa* and *reguloides* may be imaginary.

The presence of lutein in the feathers of *A. reguloides* accords with previous findings on carotenoids in birds. Generally red, orange and bright yellow colours in birds are produced by oxygenated (hydroxy and keto) derivatives of α-carotenes and β-carotenes, the so called xanthophylls (Brush 1981). The exact identification of the brown melanin (phaeomelanin) was not attempted because their exact structures are uncertain (Brush 1978).

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