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MOLECULAR ASSESSMENT OF THE TAXONOMIC STATUS OF COX'S SANDPIPER[1]

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Abstract. To determine whether *Calidris paramelanotos* is a distinct species or a hybrid, DNA sequences from the mitochondrial cytochrome-*b* gene were obtained from it, as well as from *C. ferruginea* and *C. melanotos*. The sequences of the first two taxa were identical whereas that of *C. melanotos* differed by 9%. It is argued that *C. paramelanotos* is a hybrid taxon with *C. ferruginea* constituting the maternal parent. This conclusion, combined with comparisons of protein allozyme variation effectively rule out all but one of the crosses postulated for its hybrid origin—*C. ferruginea* × *C. melanotos*.

Key words: cytochrome-b; allozymes; Charadriiformes; Cox's Sandpiper.

INTRODUCTION

Cox's Sandpiper *Calidris paramelanotos* was described from two specimens in pre-breeding moult collected at Saint Vincent's Gulf, South Australia (Parker 1982). Both specimens and other putative individuals photographed (Pringle 1987) appear to have plumage characters and measurements intermediate between those of the Pectoral Sandpiper *Calidris melanotos* and the Curlew Sandpiper *C. ferruginea* (Cox 1989). Consequently, the suggestion has been made that Cox's Sandpiper represents a hybrid between the Pectoral Sandpiper and either the Curlew Sandpiper or the Sharp-tailed Sandpiper *C. acuminata* (Cox 1989, 1990a, 1990b). However, hybrid individuals need not necessarily show characters intermediate to both parental forms (Rohwer 1994, Sibley 1994). Other species proposed to be involved in the hybrid origin of *C. paramelanotos* include the White-rumped Sandpiper *C. fuscicollis* (Buckley 1988, Cox 1989) and the Ruff *Philomachus pugnax* (Stepanyan 1990). A putative juvenile was photographed in North America (Kasprzyk et al. 1988, Vickery et al. 1988), but doubt does exist as to the identity of this bird (Cox 1990b, Monroe 1991). Vuilleumier et al. (1992) concluded that specific recognition of Cox's Sandpiper was premature, treating it as a *species inquirenda*.

To assess the taxonomic status of *Calidris paramelanotos*, we compared protein allozyme and DNA sequence variation of the mitochondrial cytochrome-*b* gene in *C. paramelanotos*, *C. melanotos* and *C. ferruginea*. Given that mitochondrial DNA is maternally inherited (Brown 1983), if either *C. melanotos* or *C. ferruginea* are involved in the origin of *C. paramelanotos*, com-

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TABLE 1. Species and sample sizes examined by protein allozymes and sequencing of the cytochrome-*b* gene. Apart from Missouri in the United States, all other localities are in Australia.

Species	Locality	Sample size	
		Allozymes	mt DNA
C. acuminata	Saint Vincent's Gulf, SA	7	—
	Bathurst, NSW	3	—
C. melanotos	Saint Vincents Gulf, SA	3	2
	Holt County, Missouri	3	1
C. paramelanotos	Saint Vincent's Gulf, SA	3	3
C. ferruginea	Saint Vincent's Gulf, SA	8	5
	Nowra, NSW	1	1
	Roebuck Bay, WA	3	1
Philomachus pugnax	Saint Vincents Gulf, SA	1	—

plete or nearly complete sequence homology would be expected between the latter and the maternal species. Such molecular data has proven useful in identifying hybridization in other birds including scrubwrens (Joseph and Moritz 1993), murres (Friesen et al. 1993) and parulid warblers (Rohwer 1994).

MATERIALS AND METHODS

Three additional specimens of *Calidris paramelanotos* were collected from Saint Vincent's Gulf over the period 1989 to 1992 and these are now housed in the Australian National Wildlife Collection (CSIRO, Canberra). Cytochrome-*b* sequences were obtained from these, as well as from three individuals of *C. melanotos* and seven individuals of *C. ferruginea* (Table 1). DNA was extracted from frozen liver samples following the protocol in Leeton et al. (1994). The cytochrome-*b* fragment was amplified using the primer pair L15114/H15547 (Edwards et al. 1991). PCR and asymmetric sequencing was performed as described in Leeton et al. (1994). DNA sequences were aligned relative to the chicken mtDNA genome (Desjardins and Morais 1990) and translated into amino acids using the computer program MEGA (Kumar et al. 1994).

Protein electrophoresis was carried out on samples of liver and skeletal muscle from *C. paramelanotos, C. melanotos, C. ferruginea* and *C. acuminata* (Table 1). Eighteen enzyme systems representing twenty-three presumptive loci were screened (acronym, Enzyme Commission Number, and number of loci scored, in parenthesis): Adenylate kinase (Ak; 2.7.4.3; 1), Aldolase (Ald; 4.1.2.13; 1), Creatine kinase (Ck; 2.7.3.2; 1), Fumarase (Fum; 4.2.1.2; 1), Glucose-phosphate isomerase (Gpi; 5.3.1.9; 1), Glutamate dehydrogenase (Glud; 1.4.1.3; 1), Glutamate oxaloacetate transaminase (Got; 2.6.1.1; 2); Glyceraldehyde-3-phosphate dehydrogenase (Ga3pd; 1.2.1.12; 1), Glycerophosphate dehydrogenase (Gpdh; 1.1.1.8; 1), Guanine deaminase (Gda; 3.5.4.3; 1), Isocitrate dehydrogenase (Idh; 1.1.1.42; 2), Lactate dehydrogenase (Ldh; 1.1.1.27; 2), Malate dehydrogenase (Mdh; 1.1.37; 2), 6-Phosphogluconic dehydrogenase (6Pgd; 1.1.1.44; 1), Phosphoglucomutase (Pgm; 2.7.5.1; 2), Phosphoglycerate kinase (Pgk; 2.7.2.3; 1), Pyruvate kinase (Pk; 2.7.1.40; 1), Triose phosphate isomerase (Tpi; 5.3.1.1; 1). Preparation of samples, electrophoretic conditions and staining procedures followed Christidis et al. (1991). Alleles and loci were designated alphabetically and numerically, respectively, according to increasing electrophoretic mobility. Material from one *Philomachus pugnax* became available after most of the allozymic analysis had been completed and was examined only for Idh and 6Pgd.

RESULTS

Sequences were obtained for a 288 bp fragment of cytochrome-*b* (Fig. 1) corresponding to the region 15377 to 15664 of the chicken mitochondrial genome (Desjardins and Morais 1990). All three *C. melanotos* examined shared a single haplotype, while single variants were observed among the individuals of *C. ferruginea* and *C. paramelanotos*. In both cases the variant involved a silent third codon transition. Sequence comparison between *C. melanotos* and *C. ferruginea* revealed 26 nucleotide base changes comprising: five transitions at codon position one, one transversion at codon position two, as well

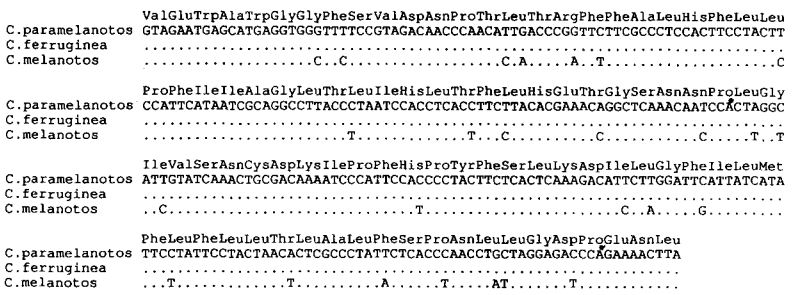

FIGURE 1. DNA sequences of a 288 bp segment of cytochrome-*b* spanning positions 15377 to 15664 (inclusive) relative to the chicken mtDNA genome (Desjardins and Morais 1990). Amino acids are listed above each codon triplet. In the *C. paramelanotos* and *C. ferruginea* sequences, asterisks denote variants in single individuals where adenine was replaced by guanine.

as 14 transitions and six transversions at codon position three. Translation of the sequences showed two amino acid changes between these species which were the result of the second codon transversion and one of the third codon transversions. Apart from the single individual variants in *C. ferruginea* and *C. paramelanotos*, these two taxa shared identical haplotypes.

Of the 23 protein loci examined, 16 were monomorphic across all species, whereas two, Ldh-1 and Pgm-1 could not be reliably scored. Among the five variable loci (Table 2), *C. paramelanotos, C. melanotos* and *C. ferruginea* were fixed for allele *a* at Mdh-1 while *C. acuminata* was fixed for allele *b*. *Philomachus pugnax* possessed allele *a* at 6Pgd which was not observed in the other species examined. *C. paramelanotos* was polymorphic at Got-2 and Idh-2, and in both cases this was due to a single heterozyote. At Got-2, the heterozygote possessed a unique allele while at Idh-2 the allellic polymorphism was shared with both *C. melanotos* and *C. ferruginea*.

DISCUSSION

Identical or near identical cytochrome-b sequences shared between *C. ferruginea* and *C. paramelanotos* supports the view that the latter represents a hybrid taxon (Cox 1989, 1990a, 1990b). The level of sequence divergence between *C. melanotos* and *C. ferruginea* (9%) is comparable to or higher than that recorded for other congeneric avian species (Edwards and Wilson 1990, Helbig et al. 1995). Within *Calidris,* the lowest level of divergence recorded in cytochrome-*b* between species is 4% (Baker, pers. comm.). Moreover, Baker (1992) and Wenink et al. (1993) reported intraspecific variation in cytochrome-*b* among individuals of *C. alpina* and *Areneria interpres.* If *C. paramelanotos* were indeed a distinct species, some sequence divergence from *C. ferruginea* would have been expected. It is therefore, reasonable to assume that the three individuals of *C. paramelanotos* we examined are the products of hybridization involving *C. ferruginea* as the maternal parent.

TABLE 2. Species examined, sample sizes and allelic frequences at polymorphic protein loci.

Locus *n*	*C. acuminata* 10	*C. melanotos* 6	*C. paramelanotos* 3	*C. ferruginea* 12	*P. pugnax* 1
Got-2	a	a	a (0.83) b (0.17)	a	—
Idh-2	b	b (0.92) c (0.08)	b (0.83) c (0.17)	a (0.04) b (0.71) c (0.25)	b
Mdh-1	b	a	a	a	—
6Pgd	c (0.25) d (0.75)	d	d	b (0.04) c (0.25) d (0.71)	a
Pgm-2	a (0.05) b (0.95)	b	b	b	—

The allozyme data are more ambiguous regarding the taxonomic status of *C. paramelanotos,* due primarily to a lack of suitable species specific markers among the taxa examined. Nevertheless, variation at Mdh-1 and 6Pgd effectively rule out *C. acuminata* and *Philomachus pugnax* as being involved in any of the hybridization events. The allelic constitutions of the three *C. paramelanotos* are consistent with a scenario in which the taxon represents a hybrid between *C. melanotos* and *C. ferruginea.* The only inconsistency is the presence of allele *b* for Got-2 in a single heterozygous *C. paramelanotos.* Given the small samples sizes of the present study, the presence of this allele in other individuals of *C. ferruginea* and *C. melanotos* cannot be ruled out.

The parental combinations that have been proposed for the hybrid origin of *C. paramelanotos* are as follows: (1) *C. melanotos* × *C. ferruginea* (Cox 1989); (2) *C. melanotos* × *C. fuscicollis* (Buckley 1988, Cox 1989); (3) *C. melanotos* × *Philomachus pugnax* (Stepanyan 1990); and *C. acuminata* × *C. ferruginea* (Cox 1989). The present allozyme and mtDNA data effectively rule out three of these combinations. Since *C. ferruginea* has to be one of the parental species, this leaves only the combinations *C. melanotos* × *C. ferruginea* and *C. acuminata* × *C. ferruginea.* Variation at the Mdh-1 locus would indicate that *C. acuminata* is not one of the parental species. Consequently, it can be concluded that the individuals of *C. paramelanotos* examined must be the result of hybridization between females of *C. ferruginea* and most probably males of *C. melanotos.*

A two dimensional scattergraph of wing length versus bill length (Fig. 2) reveals overlap between the sexes in *C. ferruginea* but pronounced sexual dimorphism in *C. melanotos.* The five specimens of *C. paramelanotos* were all originally sexed as males by dissection, though the sex of one (the paratype, SAMA B28843) is considered unproven (Cox 1987). In the scattergraph all five were treated as male. If it is assumed that *C. paramelanotos* is a hybrid and that it is intermediate in size between the parental forms, then the size distribution of the five individuals of *C. paramelanotos* are consistent only with a cross involving a female *C. ferruginea* and a male *C. melanotos* (Fig. 2). Such a scenario conforms with that deduced from the observed pattern of cytochrome-*b* variation.

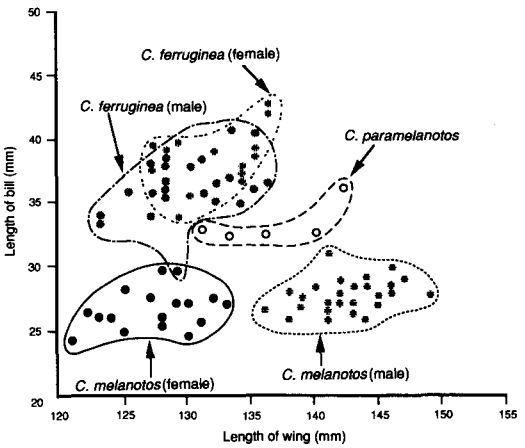

FIGURE 2. Scattergraph of lengths of wing versus bill for adult sexed specimens of *Calidris paramelanotos* (5 male), *C. melanotos* (26 male, 16 female) and *C. ferruginea* (23 male, 14 female).

Given the suggested hybrid origin of *C. paramelanotos,* it is therefore of interest that at least four of the five specimens are males. Haldane (1922) postulated that if one sex was absent or rare in the progeny of crosses between species, that sex would be usually the heterogametic sex, which in birds is the female. Hybridizations of finches and parrots in captivity does in fact corroborate this prediction, surviving males being much more the frequent sex (Gray 1958). Nevertheless, the apparent lack of female *C. paramelanotos* could equally be due to sampling error or sexual separation on the non-breeding grounds as has been recorded in *C. ferruginea* (Barter 1987).

The combination of morphological, protein allozyme, and mitochondrial DNA sequence data sets appear to show that *C. paramelanotos* is a hybrid taxon involving crosses between *C. melanotos* and *C. ferruginea.* However, the identity of other "hybrid" waders in Australia (Lane et al. 1981), their affinity with Cooper's Sandpiper (Cox 1990a, 1990b), and the identity of the hybrid recorded from Massachusetts (Cox 1990a, 1990b, Monroe 1991) remain unresolved.

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