Status of the Western Whipbird (heath subspecies): Development of Molecular Markers

Final report to

Department of Conservation and Land Management, Western Australia

November 1999

Dr Les Christidis and Dr Janette Norman
Science Program,
Museum of Victoria,
71 Victoria Crescent,
Abbotsford Victoria
Tel: 03 92840233
Fax: 03 94160475
E-mail: lchrist@mov.vic.gov.au
jnorman@mov.vic.gov.au

Introduction

Based on limited evidence and contra to other authors (eg Condon 1968), Schodde and Mason (1991) proposed that populations of western whipbirds in Western Australia (WA) be treated as two subspecies: *P. n. nigrogularis* at Two Peoples Bay - Mt Manypeaks and *P. n. oberon* at other sites in southern WA. Based on differences in size and plumage they further suggested that *P. n. oberon* was more closely aligned to the east Australian populations (*P. n. lashmari* and *P. n. leucogaster*) than to *P. n. nigrogularis*. The apparent distinctiveness of the latter led them to suggest that *P. n. nigrogularis* could represent a different species. This treatment was not followed because a number of shared characters were treated as evidence of intergradation between *nigrogularis* and *oberon*. These same characters were subsequently treated as ancestral states and *nigrogularis* accorded full species status (Schodde and Mason 1999).

Based on the taxonomy proposed by Schodde and Mason (1991), Garnett (1992) classified *P. n. nigrogularis* as endangered and *P. n. oberon* as rare. Given that only two populations of *P. n. oberon* are believed to contain more than 125 individuals, it has been suggested that this form should also be classified as endangered (Cale and Burbidge 1993). The resolution of the taxonomic status and affinities of these populations is critical for the effective management of the species. Such an assessment would assist in determining the priority level assigned to the management of the west Australian populations. If *P. n. nigrogularis* is separable from *P. n. oberon*, each would have a higher priority for research and management than if they belonged to the same subspecies.

The possibility that *P. n. nigrogularis* represents a separate species to *P. n. oberon* and the eastern forms of the western whipbird (Schodde and Mason 1991,1999) also has critical implications for management (Norman and Christidis 1997). According to this hypothesis, the intermixing of *nigrogularis* with *oberon* would produce species hybrids with unknown effects on breeding potential. It also suggests that intermixing between *oberon* and the eastern populations would be preferable to mixing *oberon* and *nigrogularis*. A further consequence is that *nigrogularis* would represent a separate species with a very limited distribution.

Molecular genetic analyses often provide more robust assessments of taxonomic relationships than possible from morphological analyses alone. The unstable taxonomy of this group clearly makes such an independent assessment warranted. Here we report the results of a preliminary molecular genetic investigation to determine the taxonomic status and affinities of the west Australian populations of the western whipbird.

Aims

The aim of this study was to develop molecular markers which would enable us to determine if *P. n. oberon* is distinct from *P. n. nigrogularis* and at what taxonomic level (species, subspecies or populations) they are separable.

The study focussed on sequence analysis of the mitochondrial NADH dehydrogenase subunit 2 (NADH-2) gene with the following objectives:

- To determine the levels of genetic differentiation characteristic of subspecies and species in the genus *Psophodes* which also includes the eastern whipbird (*P. olivaceus*), chirruping wedgebill (*P. cristatus*) and chiming wedgebill (*P. occidentalis*).
- To assess the level of genetic differentiation between populations of the western whipbird from Two Peoples Bay (*nigrogularis*) and Kangaroo Island (*lashmari*).
- To develop protocols which would enable us to use museum skins to assess the taxonomic relationships of *nigrogularis* and *oberon*.

Molecular Methods

DNA was extracted from blood or tissue samples of 10 specimens using protocols described in Norman *et al.* (1998). The sample included representatives of all four species in the genus *Psophodes* and both subspecies of the eastern whipbird (*P. olivaceus*). The western whipbird was represented by two specimens: *P. n. nigrogularis* from Two People's Bay and *P. n. lashmari* from Kangaroo Island. Details of the specimens and their geographic locations are given in Figure 1.

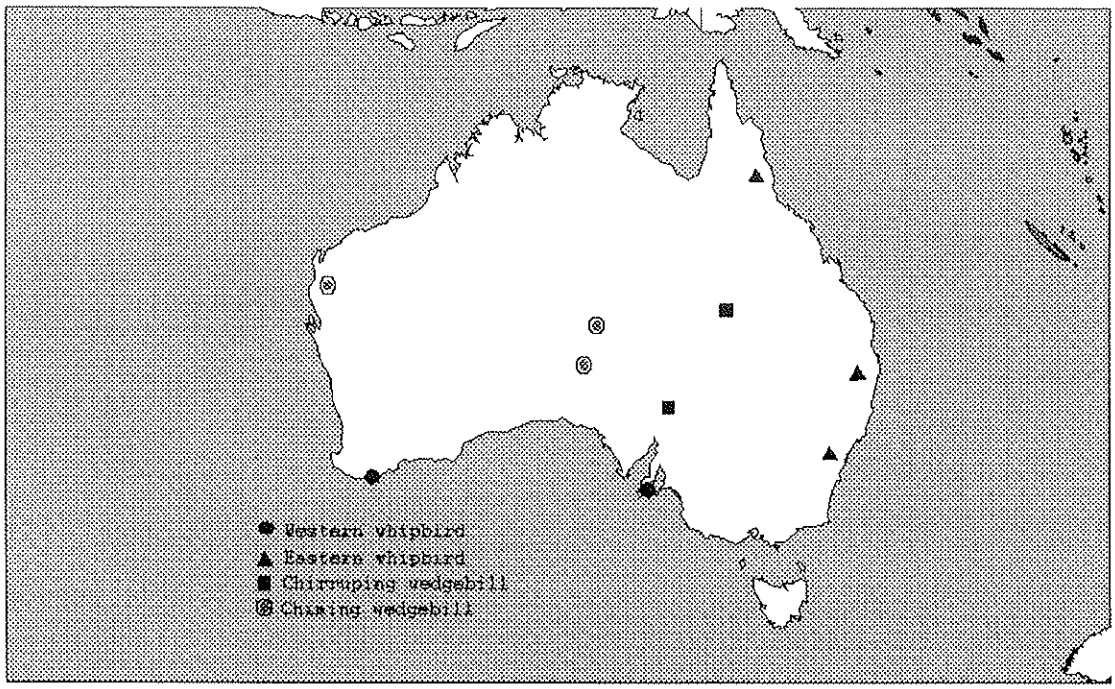

Figure 1. Geographic localities for the ten *Psophodes* specimens included in this study. Tissue numbers and sampling locations are as follows: *P. n. nigrogularis* (wwb - Two People's Bay, WA); *P. n. lashmari* (42610 - Kangaroo Island, SA); *P. o. olivaceus* (B840 - Cambridge Plateau, NSW; C291 - Mebbin State Forest, NSW); *P. o. lateralis* (Mission Beach, QLD); *P. cristatus* (D082 - Bulloo downs, QLD; MV1049 - Lyndhurst, SA); *P. occidentalis* (MV1347 - Kulgera, NT; MV2586 - Minilya, WA; W0183 - Mabel Creek, SA).

The NADH-2 gene was amplified and sequenced in all 10 specimens using primers ND2-1 and ND2-2 (provided by the Field Museum of Natural History, Chicago), amyintND2.c1 and amyintND2.nc (Norman, unpublished). Initially, the gene was amplified as a single fragment of ~1 kilobase and partial sequence obtained using primers ND2-1 and ND2-2. To obtain internal sequence it was necessary to re-amplify the fragment as two smaller products using primers ND2-1 with amyintND2.c1 and ND2-2 with amyintND2.nc. Initial PCR amplifications and

sequencing reactions were performed as described in Norman *et al* (1998). For the re-amplifications, cleaned PCR products were diluted in sterile water and subjected to PCR with a reduced extension time of 40 seconds.

The sequences were aligned and pairwise estimates of genetic divergence calculated for all specimens.

Results

A total of 1003 basepairs (bps) of NADH-2 sequence was obtained for each of four specimens. This sample comprised one western whipbird (*P. n. lashmari*) and three eastern whipbirds including representatives of both subspecies. For the remaining six specimens a total of 645 bps of sequence was obtained for each.

Uncorrected pairwise estimates of sequence divergence for the 645 bps ranged from 0.2% to 18.2%. The smallest divergences were observed among individuals within a species (for monotypic *P. cristatus* and *P. occidentalis*) or subspecies. These ranged from 0.2% to 0.6% with the greatest divergence observed between individuals of *P. cristatus*. The northern and southern subspecies of the eastern whipbird, *P. o. lateralis* and *P. o. olivaceus*, differed by 2.5% whereas the two *olivaceus* specimens differed by only 0.2%. The greatest levels of sequence divergence were observed between recognised species. These ranged from 7.5% to 18.1% with the smallest divergence observed between the morphologically similar wedgebills, *P. cristatus* and *P. occidentalis*. The eastern and western whipbirds were highly differentiated averaging

16.3% sequence divergence. This is similar to the levels of sequence divergence recorded in pairwise comparisons involving a whipbird and a wedgebill (16% to 18%). No attempt was made to account for possible saturation of the data in these comparisons.

The single specimens of *P. nigrogularis* and *P. n. oberon* differed by 1.7% sequence divergence. This is well within the range of sequence divergences recorded within a species in this genus.

Analysis of the aligned sequences showed that the variable sites were relatively evenly distributed across the gene. The only exception involved comparisons of the two forms of *P. nigrogularis*. In this case, two-fold higher levels of variation were detected in the terminal, or 3', portion of the gene. In fact, six of the nine variable sites were located within a 150 bp stretch of sequence near the 3' terminus.

An alignment of the full sequences obtained for all specimens was used to identify highly conserved regions across the length of the gene. These regions were used to design a series of ten internal primers which can be used to amplify the majority of the NADH-2 gene of the western whipbird in short 150 to 200 bp fragments (Figure 2). Using a subset of these primers the nine variable sites identified between *P. n. nigrogularis* and *P. n. oberon* can be amplified and sequenced as three separate fragments containing, one, two and six sites, respectively (Figure 2).

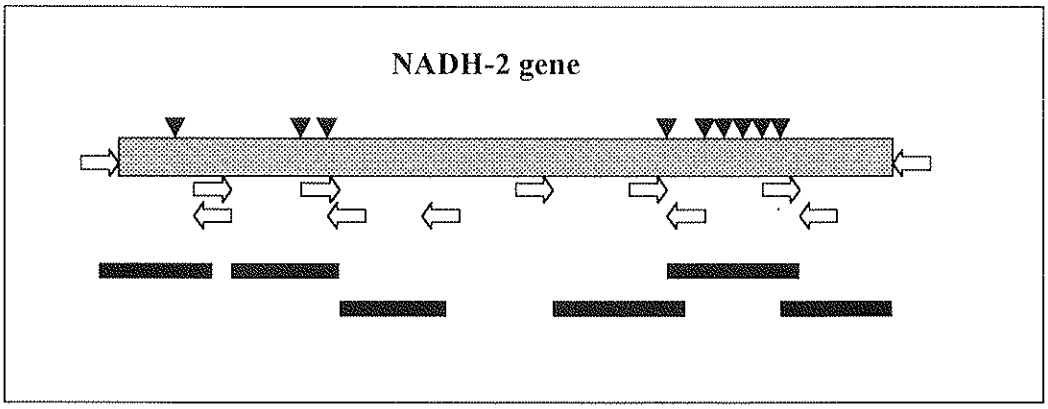

Figure 2. Location of PCR primers (arrows) designed to amplify portions of the NADH-2 gene (shaded bar) in the western whipbird. Inverted triangles indicate the location of variable sites in the sequenced portion of the NADH-2 gene in the two western whipbird samples. Solid bars indicate the approximate size and location of the fragments which potentially can be amplified from museum skins. The NADH-2 gene is 1,047 bps long.

Discussion

NADH-2 as a source of molecular markers

When using molecular approaches for taxonomic assessments it is important to analyse portions of the genome which reveal informative levels of variation. For studies of avian populations and closely related avian species, rapidly evolving portions of the mitochondrial (mt) genome appear to be the most suitable.

The results of this study indicate that the mt NADH-2 gene is an appropriate marker to use for taxonomic assessments in the genus *Psophodes*. Analysis of a 645 bp portion of this gene was sufficient to detect both intra- and inter-specific differentiation. For each species the level of divergence detected amongst individuals

(0.2% to 0.6%) was consistently less than that observed between the different subspecies of *P. olivaceus* (2.5%) or between distinct species (7.5% to 18.0%). These values are consistent with the levels of variation detected within and among other avian species (Hackett 1996; Omland *et al* 1999).

Our analyses also demonstrated that the NADH-2 gene could detect variation among geographically isolated populations of the western whipbird. A total of nine differences (1.7% divergence) separated *P. n. nigrogularis* and *P. n. lashmari*. These results further confirm the suitability of the NADH-2 gene as a molecular marker for determining the taxonomic status and affinities of the west Australian populations of the western whipbird.

Preliminary assessment of the status of P. n. nigrogularis

It is inappropriate to assess the taxonomic status of *P. n. nigrogularis* on the basis of the limited sequence comparisons performed here. Nevertheless, our preliminary data are inconsistent with the recent treatment (Schodde 1999) of *nigrogularis* as a distinct species on two grounds: the level of differentiation between the *nigrogularis* and *lashmari* specimens did not approach that observed between other allospecies in the genus *Psophodes*, nor did it exceed the level of differentiation observed between the subspecies of *P. olivaceus*.

An important caveat is that this preliminary assessment of the data is based on the assumption that the NADH-2 sequences are endogenous mtDNA in origin. Avian molecular taxonomy is complicated by the occurrence of horizontal mtDNA transfer - the transfer of mtDNA between species or subspecies as a result of hybridisation

events. Because mtDNA is maternally inherited, the hybrid offspring of such a mating inherit the mtDNA of the female parent. Historically, the populations assigned to *nigrogularis* and *oberon* have had a parapatric distribution. This increases the possibility that past hybridisation events could have resulted in the transfer of mtDNA from *oberon* to *nigrogularis*. Additional sampling and sequence analysis will be needed to confirm the origins of the mtDNA sequences obtained here.

Future research

Molecular studies of rare and threatened species are often limited by the availability of samples. In many cases non-destructive sampling methods can be used to obtain tissues (blood or feathers) for analysis. Where this is impractical museum specimens may be the only source of genetic material available. Museum specimens also provide a source of genetic material from historical localities.

Resolution of the taxonomic status and affinities of the west Australian populations of the western whipbird will require the analysis of DNA from museum specimens. The DNA in museum specimens is usually highly degraded requiring the development of modified protocols (Norman *et al* 1998). We have developed the PCR primers necessary to undertake this work.

There are a total of 23 specimens of western whipbirds in Australian museum collections. Nearly half of these are from the west Australian populations: eight from *nigrogularis* and three from *oberon*. The remaining 12 specimens are from the populations at Eyre Peninsula (1) , Yorke Peninsula (1), Kangaroo Island (5) and the Murray Mallee (4).

Summary

DNA sequence data was obtained from the mitochondrial NADH-2 gene of ten specimens. This included members of all four species in the genus *Psophodes*, both subspecies of the eastern whipbird (*P. o. olivaceus* and *P. o. lateralis*), and two representatives of the western whipbird (*P. n. nigrogularis* and *P. n. oberon*). Variation was detected at both the intra- and inter-specific level. Genetic divergences within species ranged from 0.2% to 2.5% with the maximum divergence recorded between subspecies of the eastern whipbird. Divergences between species ranged from 7.5% to 18.1%. The sequences of *P. n. nigrogularis* and *P. n. oberon* differed by 1.7%, well within the range of variation recorded within a species in this genus. From the aligned sequences a series of internal PCR primers were designed which will enable portions of the NADH-2 gene to be analysed in DNA extracted from museum specimens. The analysis of museum specimens will be necessary to determine the taxonomic status and affinities of the west Australian populations of the western whipbird.

Acknowledgments

This study was funded by CALMScience Division of the Department of Conservation and Land Management (CALM), BankWest Landscope Conservation Visa Card and Museum of Victoria. The critical tissue samples from *P. n. nigrogularis* and *P. n. lashmari* were provided by CALM Albany and the Australian National Wildlife Collection (CSIRO), respectively. Vanessa Hayes and Megan Osborne provided invaluable technical assistance.

References:

Cale, P. and Burbidge, A.H. (1993) Research Plan for the Western Ground Parrot, Western Whipbird and Western Bristlebird. Report to Australian National Parks and Wildlife Service; ANPWS ESP Project No. 228.

Condon, H. T. (1968) A Handlist of the Birds of South Australia. South Australian Ornithological Association, Adelaide.

Hacket, S.J. (1996) Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). *Molecular Phylogenetics and Evolution* **5**: 368-382.

Norman, J. and Christidis, L. (1997) Genetics and the conservation of Australian birds. *Pacific Conservation Biology*. **3**: 306-15.

Norman, J. *et al* (1998) Molecular data confirms the species status of the Christmas Island Hawk-Owl *Ninox natalis*. *Emu* **98**: 197-208.

Omland, K.E., Lanyon, S. M. and Fritz, S. J. (1999) A molecular phylogeny of the New World orioles (*Icterus*): the importance of dense taxon sampling. *Molecular Phylogenetics and Evolution* **12**: 224-239.

Schodde, R. and Mason, I.J. (1991) Subspeciation in the western whipbird *Psophodes nigrogularis* and its zoogeographical significance, with descriptions of two new subspecies. *Emu* **91**: 133-44.

Schodde, R. and Mason, I.J. (1999) The Directory of Australian Birds. Passerines. CSIRO Publishing.