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Introduction

The Grey Fantail complex consists of three species distributed across Australia, New Zealand, and Melanesia: Grey Fantail *Rhipidura albiscapa* (Australia and Melanesia), Mangrove Grey Fantail R. phasiana (Australia), and New Zealand Grey Fantail R. fuliginosa (New Zealand). A recent study of these and other fantail species, based mainly on single individuals per taxon, indicated that *R. fuliginosa* and Australian *R. albiscapa* were sister taxa, that *R. phasiana* was sister to these two, and that Melanesian *R. albiscapa* was sister to the rest of the Grey Fantail complex (Nyari et al. 2009). Thus, *R. albiscapa* was found to be polyphyletic.

Australian populations of the complex, all formerly considered conspecific, are now generally grouped into two species and six geographically isolated units: five subspecies of R. albiscapa (R. a. keasti, R. a. albicauda, R. a. preissi, R. a. alisteri, and R. a. albiscapa) and the monotypic *R. phasiana* (Fig. 1). The splitting of these two species (Ford 1981) was based largely on differences in song and habitat. Within *R. albiscapa*, the southern forms alisteri, preissi, and albiscapa were proposed to be closely related based on tail coloration, song, habitat, and clutch size (Ford 1981). In this study, we assessed genetic variation in Australian populations of the Grey Fantail complex to address the following questions:

- Does the Grey Fantail Complex show genetic variation across its Australian range and, if so, is the variation geographically structured?
- Does genetic variation correspond to morphological, behavioral, and ecological variation in these taxa? Do *R. albiscapa* and *R. phasiana* form distinct clades, and do the southern subspecies *alisteri, preissi,* and *albiscapa* form a clade?
- Alternatively, do *R. albiscapa* individuals from mainland Australia group into eastern and western Australian clades, as do some other Australian species?
- Does improved sampling within Australia change our ideas about relationships of the Australian forms to *R. fuliginosa* and Melanesian *R. albiscapa*?



R. a. albiscapa

Figure 1. Geographic ranges and sampling localities for *R. phasiana* and subspecies of *R.* albiscapa. Some points represent more than one sample. Colors correspond to species and subspecies on tree; four-point stars indicate samples that do not group with others in their geographic range. Map modified from Schodde and Mason (1999).

Patterns of genetic variation in the Australian Grey Fantail complex: Rhipidura albiscapa and Rhipidura phasiana

Materials and Methods

Thirty-eight tissue samples, representing *R. phasiana* and each of the Australian subspecies of *R. albiscapa*, were obtained from museum collections (Fig. 2; see Acknowledgments). DNA was extracted, amplified, and Sanger sequenced using standard protocols. The mitochondrial genes ND2 and ND3 were sequenced for all individuals and the nuclear intron beta-fibrinogen intron 5 (Fib5) for selected individuals. Sequences were edited and aligned in Sequencher 5.2.4. Seven additional sequences for ingroups and outgroups were obtained from Nyari et al. (2009). Maximum parsimony and maximum likelihood analyses were performed using PAUP* 4.0b10 and RAxML respectively. One thousand MP and 100 ML bootstrap replicates were conducted to assess node support for the resulting phylogenetic trees.



Figure 2. Single most parsimonious tree based on analyses of mtDNA. Numbers above branches indicate bootstrap support for mtDNA analyses (MP/ML); numbers below branches for combined analyses of nuclear and mitochndrial data (ML). Four-point stars correspond to samples specified in Fig. 1.











- sequences for 21 individuals.
- similar, although resolution was slightly reduced (Fig. 2).
- monophyletic, but were not sister taxa.
- albiscapa, making R. albiscapa polyphyletic.
- divergence of 1.3% in mtDNA.

Our results support the evolutionary distinctiveness of *R. phasiana* relative to *R. albiscapa* (Ford 1981, Nyari et al. 2009) and the polyphyly of *R. albiscapa* when Melanesian subspecies *R. a. brenchleyi* is included (Nyari et al. 2009). Within *R. albiscapa*, the southern forms *alisteri, preissi,* and *albiscapa* are not sister taxa (*contra* Ford 1981) but instead span the entire genetic diversity of Australian *R. albiscapa*. Thus, genetic variation is congruent with phenotypic variation in supporting species status of *R. phasiana*, but incongruent with phenotypic variation in Australian populations of *R. albiscapa*.

The exceptions to the geographical patterns in *R. albiscapa* are likely due to different factors. An east-west genetic break near the Nullarbor Plain is roughly consistent with that in some other Australian species, such as Musk Duck *Biziura lobata* (Guay et al. 2010); however, the shift of the break slightly west of the Nullarbor may indicate dispersal across the Nullarbor, introgression of *alisteri* mtDNA into *preissi*, or lack of lineage sorting. In contrast, the individual from the range of *alisteri* that grouped with *albiscapa* appears to be a wintering migrant. This sample was collected in late August (winter) within the known wintering area of migrant *albiscapa* and the skin has since been identified as albiscapa (R. Faucett, pers. comm.).

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Literature Cited

Ford, J. 1981. Evolution, distribution and stage of speciation in the *Rhipidura fuliginosa* complex in Australia. Emu 81: 128-144. Guay, P.-J., R. T. Chesser, R. A. Mulder, A. D. Afton, D. C. Paton, and K. G. McCracken. 2010. East-west differentiation in Musk Ducks (*Biziura lobata*) suggests late Pleistocene divergence at the Nullarbor Plain. *Conservation Genetics* 11: 2105-2120. Nyari, A.S., B.W. Benz, K.A. Jonsson, J. Fjeldsa, and R.G. Moyle. 2009. Phylogenetic relationships of fantails (Aves: Rhipiduridae). Zoologica Scripta 38: 553-561. Schodde, R. and I. J. Mason. 1999. *Directory of Australian Birds, Vol. 1: Passerines*. CSIRO Publishing Collingwood, Australia.



Results

Complete ND2 and ND3 sequences were obtained for all 38 individuals and Fib5

Trees based on mtDNA were well resolved; trees based on the combined data were

• *R. phasiana* and Australian populations of *R. albiscapa* were each found to be

• Melanesian *R. a. brenchleyi* was not closely related to Australian populations of *R.*

• Sequence variation within Australian *R. albiscapa* was low, with a maximum sequence

• With three exceptions, Australian *R. albiscapa* grouped into three taxonomically and geographically coherent clades: (1) *albiscapa* (Tasmania), (2) *keasti* and *alisteri*

(eastern Australia), and (3) *preissi* and *albicauda* (western Australia).

• Two of the three easternmost *preissi* individuals grouped with *keasti* and *alisteri*, and one individual from the range of *alisteri* grouped with *albiscapa*.

• An east-west genetic divide was observed near the Nullarbor Plain, a known geographic barrier, but was shifted slightly west of the Nullarbor.

Discussion

Acknowledgments