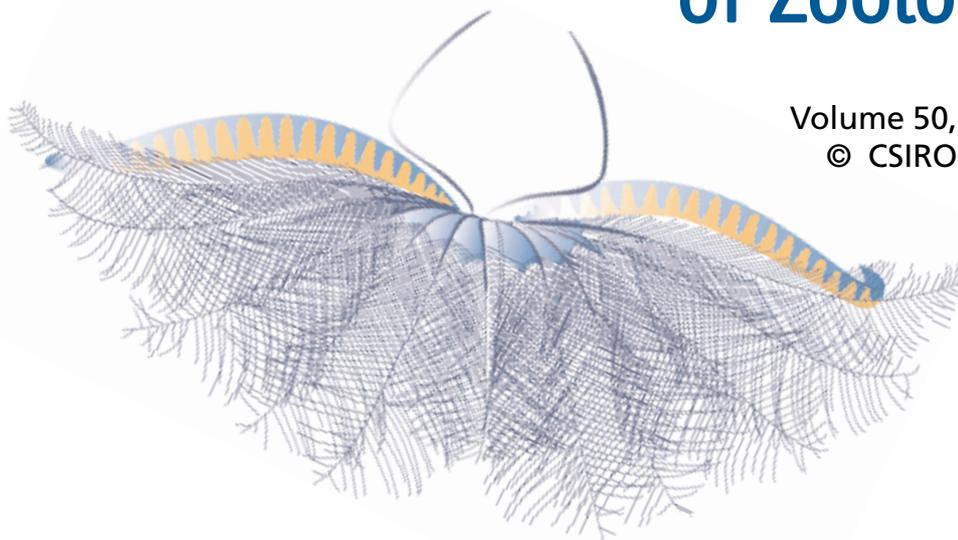


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# Australian Journal of Zoology

Volume 50, 2002  
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## Evolutionary relationships among blue- and black-plumaged populations of the white-winged fairy-wren (*Malurus leucopterus*)

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### Abstract

The white-winged fairy-wren (*Malurus leucopterus*) exhibits striking plumage colour variation between the Australian mainland and two islands (Dirk Hartog Island and Barrow Island) off the coast of Western Australia. Adult males on the mainland are bright blue with white wings and adult males on the two islands are black with white wings. To examine evolutionary relationships within this species, we sequenced 980 base pairs of two mitochondrial genes from 34 individuals from both islands and five mainland sites. Birds on Barrow Island were the most genetically distinct. Specimens from Dirk Hartog shared a unique character with, and were most similar to, birds from the Western Australian mainland. The black-and-white-plumaged subspecies from the two islands were not each other's closest relatives. Mapping of plumage evolution produced two equally parsimonious hypotheses: (1) black plumage arose from blue plumage convergently on the two islands, or (2) black plumage arose from blue plumage once and was followed by a re-evolution of blue plumage in mainland Western Australia birds. Levels of genetic differentiation in this species were low but genetic differentiation was discovered between morphologically identical eastern and western populations of the mainland subspecies, which is evidence for a current barrier to gene flow on mainland Australia.

### Introduction

By seeking to understand and account for geographic variation, evolutionary biologists hope to gain insight into evolution and the speciation process. It is generally assumed that observed morphological variation will be associated with similar and parallel genetic variation, but a number of recent studies of passerine birds have failed to find significant amounts of genetic differentiation among morphologically distinct populations (Zink and Dittmann 1993; Greenberg 1998; Piertney *et al.* 2001). This uncoupling of genetic and morphological variation may be due to the morphological variation being environmentally induced and largely non-genetic (James 1983) or because morphological differentiation has occurred so rapidly and recently that measurable genetic differentiation has not yet arisen (Orr and Smith 1998).

An excellent system for examination of the link between morphological and genetic variation is the islands off the Australian coast, which are home to a number of morphologically distinct subspecies (Ford 1987; Schodde and Mason 1999). In most cases, the island subspecies differ from their mainland counterparts by size and other morphometric differences, with no or only slight plumage differences (Keast 1961; Abbott 1974; Ford 1987; Schodde and Mason 1999). The most dramatic exception to this generality is the white-winged fairy-wren (*Malurus leucopterus* Dumont), distributed

throughout drier regions of the mainland and on two islands off the west coast of Western Australia, Dirk Hartog Island and Barrow Island.

First described from Dirk Hartog Island, the white-winged fairy-wren exhibits two distinct plumage forms (Schodde 1982*b*; Rowley and Russell 1997; Schodde and Mason 1999). In the mainland subspecies, *M. l. leuconotus*, adult males in breeding plumage are cobalt blue with white wings. In contrast, in two endemic island subspecies, *M. l. leucopterus* from Dirk Hartog Island, and *M. l. edouardi* from Barrow Island, adult males in breeding plumage are satin black with white wings. Females of each of the three subspecies are almost entirely fawn to greyish-brown in colouration.

Other than the differences in male colouration, and slight morphological differences (Rowley and Russell 1997), aspects of life history and social behaviour of the different subspecies are strikingly similar, at least when the birds on Barrow Island and those on the mainland (Rowley and Russell 1995, 1997; Pruett-Jones and Tarvin 2001) are compared. Nevertheless, the colour variation in adult males between the mainland and the two island subspecies is important, particularly in light of the location and geological history of Dirk Hartog and Barrow Islands. Variation in sea levels during the Pleistocene connected the islands to the mainland via land bridges. Each island is closer to the mainland (2 km for Dirk Hartog Island and 56 km for Barrow Island) than they are to each other (approximately 600 km), and during periods of lower sea levels any association between island populations would have been primarily via their links to the mainland.

Ford (1987) argued that two possible hypotheses could explain the colour variation in *M. leucopterus*. The first possibility is that the two island subspecies evolved their black nuptial plumage independently but convergently from a blue and-white mainland ancestor. Alternatively, the two island subspecies could be remnant populations of a previously more widely distributed black-and-white form, now extinct on the mainland (Ford 1987). Ford considered the second, remnant, hypothesis more likely as the primarily black-plumaged species *M. melanocephalus* and *M. alboscapulatus* are closely related to *M. leucopterus*, and therefore the ancestor of *M. leucopterus* was likely black-plumaged as well. Recent biochemical and molecular evidence establishes *M. leucopterus* as a member of the black-plumaged clade of fairy-wrens (Christidis and Schodde 1997; Driskell and Pruett-Jones, unpublished data), lending credence to Ford's assumptions. In addition to Ford's (1987) two hypotheses, another possible evolutionary scenario is that blue plumage in mainland *M. leucopterus* evolved more than once from a widespread black-and-white ancestor.

These competing hypotheses can be tested using genetic data. If the two island populations evolved independently from a blue-and-white ancestral population, each island should be genetically more similar to the mainland population than to each other. Fairy-wrens are generally weak flyers, and gene flow between the two islands has likely been non-existent at least since the sea levels rose after the last ice age. Alternatively, if the island populations are remnant populations of a previously widespread black-and-white form, the island populations should be more similar genetically, even with little or no gene flow between them, than either is to the mainland population. Lastly, if blue plumage evolved more than once on the mainland, some blue-plumaged populations should be more closely related to black-plumaged populations than to other blue-plumaged populations.

In this study, we examined genetic variation and phylogenetic relationships among blue- and black-plumaged populations of all three subspecies of *M. leucopterus* using mitochondrial loci. Our goal was to determine the level of genetic differentiation between

black- and blue-plumaged forms and to test the hypotheses regarding plumage evolution in this species.

## Methods

### *Samples*

Tissue samples from 34 specimens of *Malurus leucopterus*, including samples of eight individuals from Barrow Island and 10 from Dirk Hartog Island were obtained for sequencing either through our fieldwork (Pruett-Jones and Tarvin 2001) or through collaborators (Appendix 1). In addition, one sample each from *M. lamberti* and *M. melanocephalus* was used for outgroup analysis.

### *Molecular techniques*

DNA was extracted from ethanol-preserved tissue or blood using standard proteinase-*k* digestion followed by either phenol–chloroform extraction or the protein-precipitation method as implemented in the Puregene kit (Gentra Systems, Inc.). Each gene region was amplified in a single PCR reaction following standard protocols. The primers used for both amplification and sequencing of the mitochondrial NADH dehydrogenase subunit 3 gene (ND3) were L10755 AND H11151 (Chesser 2000) and the mitochondrial ATP synthase subunit 6 gene (ATP6) were CO3HMH and A8PWL (Seutin and Bermingham, unpublished; see Acknowledgments). Cycle sequencing of both strands of all PCR products was accomplished with the Big Dye Termination Mix (Applied Biosystems Inc.), following published protocols. Sequencing products were visualised using an ABI 377 Automated Sequencer.

All output from the automated sequencer was checked by hand for accuracy. Multiple sequences of a given gene from an individual were compiled using the program Sequencher 3.1 (Genecodes). Upon completion of sequencing, a single ‘consensus’ sequence for each individual was produced and imported into PAUP\* 4.0b10 (Swofford 2002) for alignment and analysis. Nuclear copies of a number of mitochondrial gene regions (*numts*) have been identified in previous avian studies (reviewed by Quinn 1997). Although it is impossible to completely guard against the amplification of *numts* and it is sometimes impossible to identify them in a data set, a number of precautions were taken. Sequences were carefully checked for the presence of ‘double’ peaks such as would result from the mixed amplification of a nuclear and mitochondrial target. In addition, the sequence data for every individual were translated and checked for termination codons and the data were aligned to published sequences.

### *Phylogenetic analyses*

All phylogenetic analyses, except Bayesian analysis, were conducted using PAUP\* 4.0b10 (Swofford 2001). An unweighted heuristic search with 100 random addition sequences and tree-bisection–reconnection branch-swapping (TBR) was performed under the parsimony criterion. Nodal support was evaluated with 100 replicates of bootstrapping, each with two random addition sequences. A neighbour-joining analysis (Saitou and Nei 1987) was conducted on a matrix of HKY85 (Hasegawa *et al.* 1985) distances. Prior to likelihood analysis, MODELTEST 3.0 (Posada and Crandall 1998) was used to determine the most parameter-rich model justified by the data, and to estimate likelihood parameters. A heuristic search under the likelihood criterion using the justified model with 10 random addition sequences was then undertaken, followed by 200 replicates of bootstrapping using the same model. In all of the above tree searches, the branch-swapping portion would have been greatly prolonged due to the identity or near-identity of many of the sequences in the data set; therefore branch-swapping was limited to 30 minutes per replicate.

Bayesian Markov Chain Monte Carlo (MCMC) analysis using MR BAYES ver. 1.11 (Huelsenbeck 2000) was employed as another method to estimate confidence intervals on nodes. Four Bayesian MCMC analyses were run for 1 000 000 generations each, with sampling of trees and parameters every 500 generations. Visual inspection of plots of likelihood by generation revealed that the Markov chains appeared to converge on the target distribution within the first 50 000 generations. However, we discarded the first 200 trees from each run to ensure that the chains had reached stationarity. Bayesian posterior probabilities for each node were produced by calculating a majority-rule consensus from the combined remaining trees (Larget and Simon 1999; Huelsenbeck and Bollback 2001). The maximum *a posteriori* (MAP) estimate of the topology was taken to be the tree with the highest overall likelihood from all 4 000 000 generations.

**Table 1.** Average uncorrected genetic distances within and among samples of white-winged fairy-wrens (*Malurus leucopterus*)

Values along diagonal are the average within-population uncorrected genetic distance. Values in parentheses are standard deviations

	Barrow Island	Dirk Hartog Island	Western Australia	Eastern Australia
Barrow Island	0.13% (0.07)			
Dirk Hartog Island	0.48% (0.10)	0.22% (0.16)		
Western Australia	0.60% (0.07)	0.29% (0.14)	0.07% (0.06)	
Eastern Australia	0.54% (0.12)	0.43% (0.17)	0.44% (0.13)	0.28% (0.16)

#### Other analyses

In order to determine whether white-wing populations have a history of population growth, decline or stability, we calculated a number of statistics using DnaSP ver. 3.0 (Rozas and Rozas 1999). We calculated Tajima's (1989)  $D$ , Fu and Li's (1993)  $F^*$  and  $D^*$ , and Fu's (1997)  $F_s$ . Fu's  $F_s$  test detects the excess of rare (i.e. young) alleles characteristic of a growing population, and, in combination, these four statistics can be used to indicate the likely mechanism for the observed level of polymorphism in a population (Fu 1997). In addition, we used the frequency distribution of the number of pairwise differences (i.e. mismatch distribution) among the individuals in a population (Rogers 1995) to examine whether the levels of observed polymorphism better fit hypotheses of recent population expansion or population stability (Federov and Stenseth 2001; Joseph *et al.* 2002).

A minimum-spanning tree illustrating phylogenetic relationships among all observed haplotypes was created by hand from an unrooted neighbour-joining phylogram and a matrix of uncorrected genetic distances. Plumage evolution was mapped as a discrete character onto four possible topologies arising from the phylogenetic analyses using the parsimony criterion as implemented in MacClade 3.07 (Maddison and Maddison 1992).

## Results

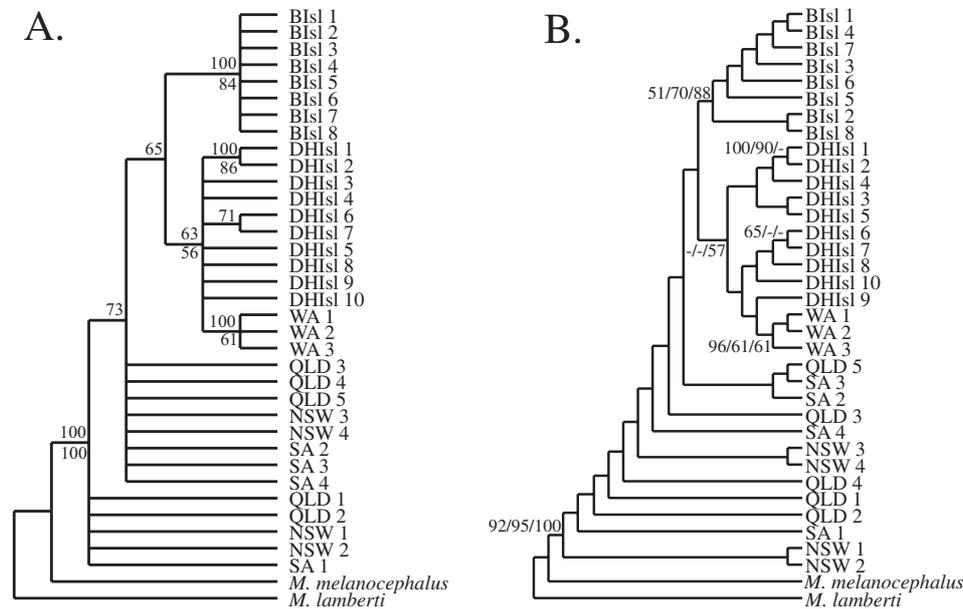
### Sequence variability

Our data set consisted of 1029 base pairs (bp) of DNA sequence data that included 351 bp of ND3 and 678 bp of ATP6. All sequences were deposited into Genbank under the accession numbers AY192072–AY192143. The entire data set comprised 181 variable nucleotide positions, of which 48 were potentially parsimony informative. Among the *M. leucopterus* specimens there were 26 variable characters, of which 10 were potentially parsimony informative. The ND3 sequence was more variable than the ATP6 sequence (3.7% vs 2.0%). There was only one second-codon position change among the *M. leucopterus* specimens (in ATP6) and the ratio of first-codon position changes to third-codon position changes was 1:2 for ND3 and 3:11 for ATP6. The ratio of transitions to transversions was 23:5.

Calculated across both genes, uncorrected genetic divergences among the *Malurus leucopterus* specimens ranged from 0.0 to 0.82%. The average divergence between *M. leucopterus* and *M. lamberti* was 12.3% and between *M. leucopterus* and *M. melanocephalus* was 8.1%. Specimens of *M. leucopterus* from Dirk Hartog Island and mainland Western Australia (WA) were the most closely related, while the Barrow Island samples showed the greatest divergence from all others (Table 1). The Dirk Hartog and mainland WA samples were essentially equidistant from the eastern Australia sample.

### Phylogenetic analysis

The search under the parsimony criterion produced 17 082 equally parsimonious trees (EPTs). Tree statistics for these trees were as follows: length = 187 steps, C.I. = 0.93,



**Fig. 1.** (A) A majority-rule consensus of all 17082 equally parsimonious trees. Numbers above the nodes are the percentage of trees in which that node is found. Numbers below the nodes are parsimony bootstrap proportions. Where no bootstrap value is indicated, the node received <50% bootstrap support. Tree statistics: length = 187, C.I. (excluding uninformative characters) = 0.77, R.I. = 0.86, R.C. = 0.80. (B) The maximum *a posteriori* estimate of the likelihood topology obtained during Bayesian MCMC analysis. Numbers at the nodes are Bayesian posterior probabilities/maximum-likelihood bootstrap proportions/neighbour-joining bootstrap proportions. A dash (-) indicates a node that received <50% support. lnL = -2199.85.

R.I. = 0.075, R.C. = 0.80. The strict consensus of these trees (not shown) retained only a monophyletic *M. leucopterus*, a Barrow Island clade, and a mainland WA clade. A majority-rule consensus (Fig. 1a) of the EPTs preserved more structure: a clade containing the Dirk Hartog and mainland WA specimens was present in most of the EPTs, although it received low bootstrap support. In addition, the majority-rule consensus showed that a clade containing both island populations and the mainland WA specimens was present in the majority of the EPTs, but this was not supported with bootstrapping. The neighbour-joining (NJ) topology (not shown) was completely congruent with the majority-rule consensus topology and depicted a well supported Barrow Island clade, a poorly supported mainland WA clade, and a poorly supported Dirk Hartog + mainland WA clade (NJ bootstrap support values for these clades indicated on Fig. 1b).

The MODELTEST analysis (Posada and Crandall 1998) indicated that a HKY model (Hasegawa *et al.* 1985) with a gamma parameter to estimate the variation in substitution rates among sites was the maximum-likelihood model best suited to the data. The model had the following parameters: 4.82 (ti/tv ratio), 0.2538 (alpha), and 0.2881/0.3478/0.1042/0.2599 (frequency of A/C/G/T). Seven equally likely trees resulted from the analysis (lnL = -2190.75) and the strict consensus likelihood (ML) topology was less resolved than, but congruent with, the parsimony majority-rule and NJ topologies. Unlike the previous two topologies, the ML topology had a polychotomy involving the monophyletic Barrow Island and mainland WA clades and the Dirk Hartog specimens.

Only two nodes of interest were well supported by bootstrapping: a monophyletic *M. leucopterus* and a monophyletic Barrow Island clade (Fig. 1*b*). The maximum-likelihood search recovered four additional trees only 0.10 log-likelihood units less likely than the seven most likely trees. These four trees were identical to the parsimony majority-rule topology (Fig. 1*a*) and the Bayesian MAP topology (Fig. 1*b*), in which the Dirk Hartog specimens formed a monophyletic group with the mainland WA sample. An SH-test (Shimodaira and Hasegawa 1999) of the seven most likely trees and the four trees with slightly lower likelihood produced no significant results, indicating that these 11 topologies are essentially equally likely. The MAP topology obtained via Bayesian MCMC analysis (Fig. 1*b*) was congruent with all other topologies. Only the mainland WA clade, however, received a statistically significant posterior probability.

To summarise the phylogenetic results: all analyses produced a monophyletic clade consisting of all island and western mainland specimens, although this grouping received no bootstrapping support. All analyses returned a topology with a moderately to well supported monophyletic Barrow Island clade. This clade was further supported by the presence of two synapomorphies in the sequence data: position 245 in ATP6 and position 246 in ND3, both third-position transitions. In addition, the three specimens from mainland WA formed a monophyletic group, which received relatively poor bootstrap support but a high posterior probability (and was present in all 17082 equally parsimonious trees). This group was supported by one synapomorphy: position 623 in ATP6, a third-position transition. A clade comprising the Dirk Hartog specimens and the Western Australia clade was found in the parsimony majority-rule consensus, the NJ, and the MAP topologies, as well in four trees arising from likelihood search that were not significantly less likely than the most likely trees. Although this clade was not strongly supported by either the Bayesian posterior or bootstrap proportions, it was supported by one synapomorphy (position 74 in ATP6), a third-position transition. There was no apparent geographic structure among the specimens from Australia east of the Nullarbor Plain; haplotypes from specimens from the same collection locality were not necessarily most closely related and there were no strongly supported clades among these specimens.

#### *Other analysis*

Only the Barrow Island, Dirk Hartog Island, and eastern Australian samples were subjected to separate intrapopulation analyses, as the mainland WA sample was too small to calculate most population statistics. None of the values of Tajima's *D*, *F<sub>u</sub>* and Li's *F\* and D\** for any of the three populations was significant (Table 2). But the Barrow Island sample had a significant value for *F<sub>u</sub>*'s *F<sub>s</sub>*, and both the eastern Australian and Dirk Hartog Island samples were very nearly significant. The mismatch distribution analyses (Fig. 2) provided additional evidence that all three populations have undergone recent expansion: expected pairwise differences fit a population-growth model better than a population-stability model.

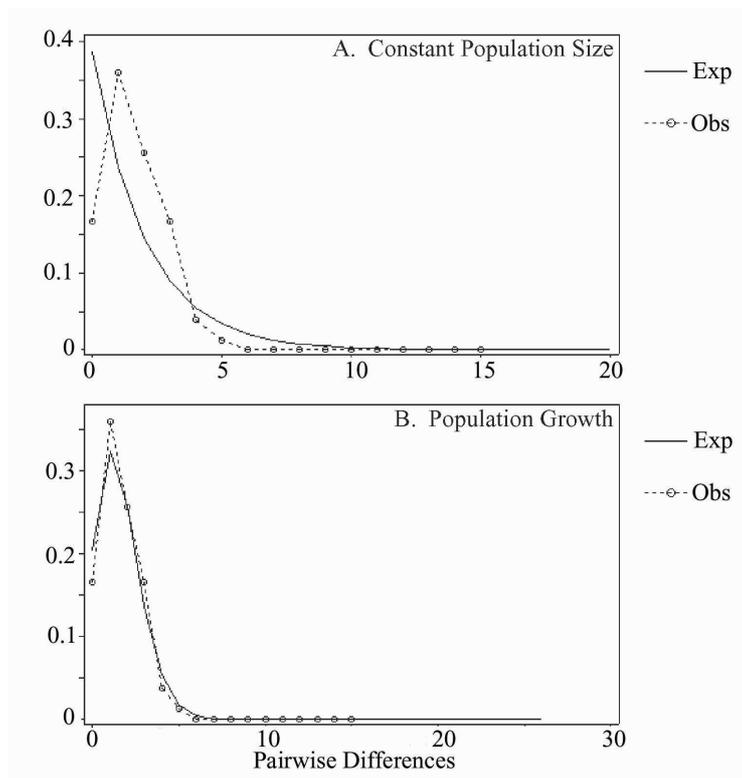
The minimum-spanning network (Fig. 3) for the *M. leucopterus* specimens showed the Barrow Island population as distinct from the other specimens, the mainland WA sample as a distinct lineage most closely related to some of the Dirk Hartog specimens, and most Dirk Hartog specimens (samples 1–8) as more closely related to one another than to any other samples.

Male nuptial plumage colouration was mapped onto two different phylogenetic hypotheses (Fig. 4), which represent the possible resolution of a critical polytomy in our

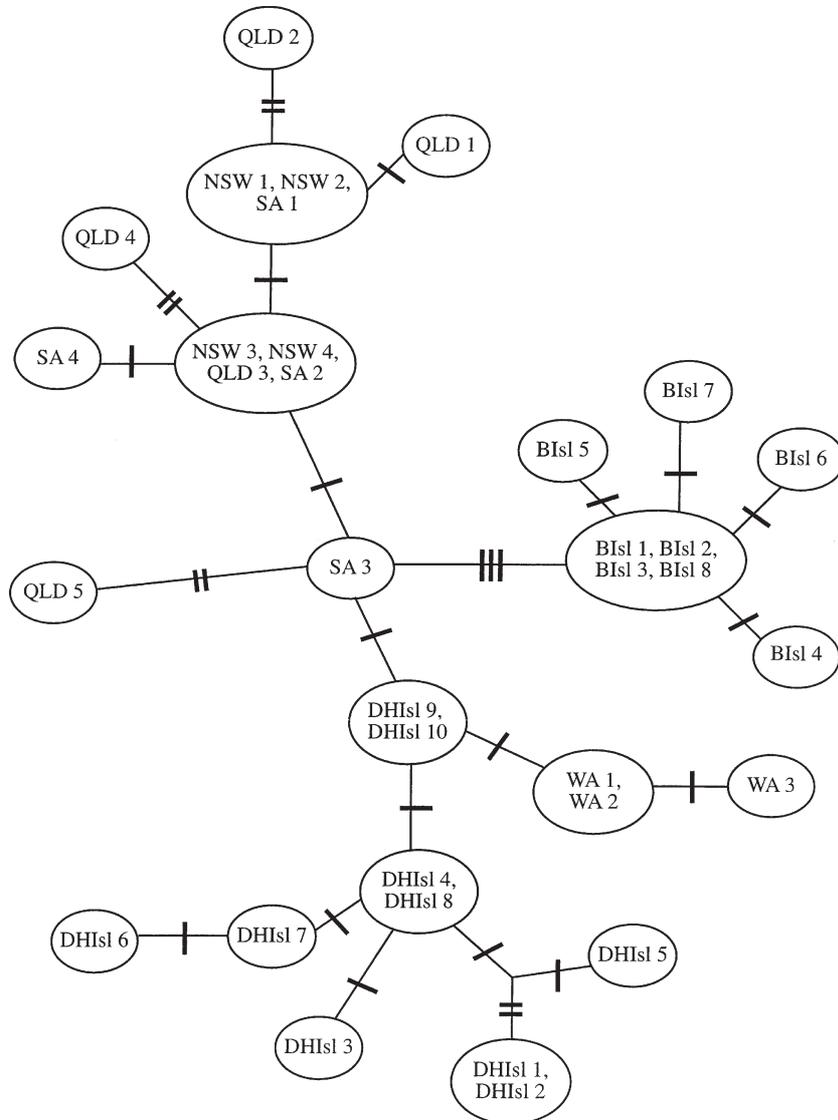
**Table 2. Divergence and population statistics for the Eastern Australia sample, and the Barrow Island and Dirk Hartog Island, WA, samples of *M. leucopterus***

Both gene regions were pooled for these calculations. Significant values are in bold and provide evidence for range expansion in Barrow Island. Sources for the statistics are as follows: D (Tajima 1989),  $F^*$  and  $D^*$  (Fu and Li 1993),  $F_S$  (Fu 1997). Abbreviations are as follows:  $N$  = sample size,  $N_{hap}$  = number of unique haplotypes,  $N_{poly}$  = number of polymorphic sites

	Eastern Australia	Barrow Island	Dirk Hartog Island
$N, N_{hap}, N_{poly}$	13, 7, 8	8, 5, 5	10, 4, 3
Nucleotide diversity (%)	0.16	0.13	0.08
Tajima's D	-1.48, $P > 0.10$	-1.53, $0.10 > P > 0.05$	-1.03, $P > 0.10$
Fu & Li's $F^*$	-2.18, $0.10 > P > 0.05$	-1.80, $P > 0.10$	-0.96, $P > 0.10$
Fu & Li's $D^*$	-2.07, $1.10 > P > 0.05$	-1.67, $P > 0.10$	-0.80, $P > 0.10$
Fu's $F_S$ (95% conf. interval)	-3.04 (-3.64, 4.39)	<b>-2.80</b> (-2.38, 3.75)	-1.47 (-1.96, 3.39)

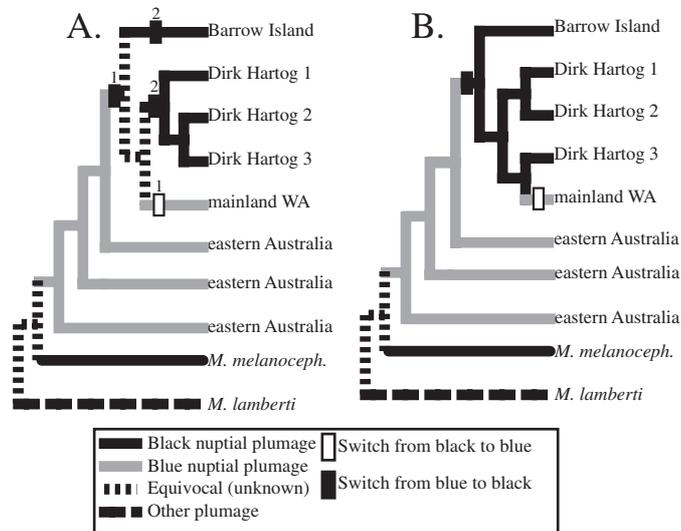


**Fig. 2.** Mismatch distributions for eastern Australian samples of *M. leucopterus* compared with expectations (A) under a model of constant population size, and (B) under a model of expanding population size. The excess of rare alleles in this sample is better approximated by the population-growth model. The mismatch distributions of the Barrow Island and Dirk Hartog Island samples were very similar to that depicted here and similarly better fit the population-growth model.



**Fig. 3.** An unrooted haplotype network for the *M. leucopterus* specimens based on a neighbour-joining (Saitou and Nei 1987) analysis of HKY85 (Hasegawa *et al.* 1985) distances. Specimens within the same oval have identical sequences. Ovals are connected to the next most similar sequence. Hash marks along the edges connecting ovals indicate the number of sequence differences between sequences.

phylogenetic results. As indicated by all phylogenetic analyses, the western birds were depicted as a monophyletic clade, nested within the eastern Australian specimens. Therefore, blue nuptial plumage was assumed to have arisen in the ancestor of *M. leucopterus*. Two different evolutionary scenarios arose from this mapping. Both scenarios posit two evolutionary changes in nuptial plumage subsequent to the origin of *M. leucopterus*, and arise if the mainland WA specimens are resolved as sister to all or part of the Dirk Hartog specimens (Fig. 4A). Either black plumage evolved in the ancestor of all western birds and



**Fig. 4.** Two possible resolutions of the key polytomy arising in phylogenetic analysis. The 10 Dirk Hartog specimens are divided into three different taxa for illustrative purposes. All specimens from Queensland, New South Wales and South Australia are depicted as three groups identified as 'eastern Australia' although more than three lineages are likely present among the eastern specimens. Male nuptial plumage is mapped onto the topologies using the parsimony criterion. Hypothesised evolutionary changes are indicated by black and white boxes. Where two equally parsimonious sets of changes are hypothesised, changes in each set are numbered.

was followed by a re-evolution of blue plumage in the mainland WA birds, or black plumage evolved twice, in parallel, in the two island populations. If the mainland WA birds are nested within all or part of the Dirk Hartog specimens (Figs 4B), only the first scenario arises: black plumage evolved in the ancestor of western *M. leucopterus* and the ancestor of mainland WA birds re-evolved blue nuptial plumage. If the two island populations form a monophyletic group to the exclusion of the mainland WA specimens, a result that did not obtain from our phylogenetic analyses, then only one evolutionary change is required: a change from blue to black nuptial plumage in the ancestor of the island populations. However, under the parsimony criterion, forcing all island black specimens to form a monophyletic group increases tree length by only one step.

### Discussion

The level of genetic differentiation between black-plumaged and blue-plumaged populations of white-winged fairy-wrens was small, and is on the order of what has been observed between many populations of passerine species (Klicka and Zink 1997). Given the extreme difference in male nuptial plumage between island and mainland white-wings, a concomitant large genetic divergence was expected but not observed. The low level of genetic differentiation indicates that the shift from blue to black, or black to blue, male nuptial plumage was relatively rapid and recent. It is conceivable that the change in plumage colour is under the control of a single genetic switch, as has recently been

discovered in the Caribbean bananaquit (*Coereba flaveola*: Theron *et al.* 2001), but this remains to be explored.

On the basis of our analyses, however, the phylogenetic hypothesis for *M. leucopterus* that requires only a single evolution of black plumage from blue (where the two island populations form a monophyletic group), is one that is not consistent with our data. It is more likely, therefore, that the story of plumage evolution in *M. leucopterus* is more complicated, and entails either (1) parallel evolution of black plumage from blue in both island populations, or (2) the evolution of black from blue in the ancestor of all western populations, followed by the subsequent re-evolution of blue plumage in the ancestor of the mainland WA birds. Neither of these possible evolutionary scenarios is completely consistent with Ford's (1987) postulation about a widespread mainland black-and-white ancestor. Unfortunately, our current data set does not completely resolve the phylogenetic relationships among white-wing populations and therefore we cannot presently choose between these two possible evolutionary scenarios.

Although relationships among the different populations are not completely resolved in our analysis, we can confidently conclude that the Barrow Island and mainland WA populations are genetically isolated from all other populations examined. There is little or no support for a monophyletic Dirk Hartog clade. The Dirk Hartog specimens shared a fixed difference with, and, in terms of genetic distance, were most similar to, the mainland WA samples. However, the mainland WA birds were distinct and the relationship between the mainland WA and Dirk Hartog birds was only weakly supported. All analyses indicated that the two island populations and the mainland WA sample form a monophyletic group nested within the sample of eastern mainland birds, although this received no bootstrap support. We believe the lack of support for many of the relationships is largely attributable to the close genetic similarity of the specimens and the very low number of phylogenetically informative characters in the data set, rather than any inherent conflict in the data. Homoplasy in the data set was low (H.I. for the parsimony topologies was 0.075). The mainland WA clade and the Dirk Hartog + mainland WA clades were each supported by an unambiguous synapomorphy, but these single characters fell short of the minimum number needed to achieve statistical significance via bootstrapping (Felsenstein 1985). Therefore, in all analyses these nodes received bootstrap support of only approximately 60%.

On the basis of genetic distances (and fixed sequence differences) the mainland WA and Dirk Hartog birds have interbred much more recently than have mainland WA and Barrow Island birds. The Dirk Hartog and mainland populations shared one fixed difference (G at position 74 in ATP6) but another (T at position 623 in ATP6) was unique to the mainland WA sample. This suggests that the two populations interbred at some point in the past, but since the cessation of gene flow sufficient time has passed for the mainland WA population to evolve another unique character. Of course, it is possible that the unique mainland WA character is present at low frequency on Dirk Hartog and that our sample of 10 specimens was not large enough to detect it. If this character is later shown to occur in the Dirk Hartog population, this would be consistent with a scenario in which mainland birds rarely, but occasionally, are vagrant on the island and interbreed with the island population. Although fairy-wrens are generally weak flyers, a gap of only 2 km separates Dirk Hartog from the mainland, and individual fairy-wrens may move between these two areas. Hybrid specimens of blue-and-white and black-and-white birds have never been collected (Schodde and Mason 1999), but there are occasional reports of black-plumaged white-winged fairy-wrens (presumably originating from Dirk Hartog Island) seen on the mainland opposite Dirk Hartog Island (Collins 1995).

If, for some reason, the vagrants between Dirk Hartog Island and the mainland were only male birds, we would be unable to detect interbreeding between the mainland and island populations using our current data set due to the matrilineal inheritance of mitochondrial DNA. Occasional mixing of a mainland male into the island population, or vice versa, would leave the maternal lineage undiluted and we could, therefore, not observe the mainland WA haplotype on the island. However, in fairy-wrens, it is usually females that disperse the farthest from their natal territory (Rowley and Russell 1997) and therefore females might be more likely to disperse between the two populations.

An unexpected finding of this study was the apparent isolation of mainland WA *M. leucopterus leuconotus* from conspecifics in the eastern portion of the species' range. Range maps for the species show an uninterrupted distribution across the entire continent (Schodde and Mason 1999). White-winged fairy-wrens can be observed continuously from east to west across its distribution, but breeding records are discontinuous (Blakers *et al.* 1984). Although our mainland WA sample consisted only of three specimens from the western part of WA, our eastern sample was relatively large and covered a broad geographic range. None of the eastern specimens had either of the distinguishing genetic characteristics of the western birds. The dissimilarity of eastern and western birds implies a significant period of isolation. Our current samples are not large enough to pinpoint the exact zone of discontinuity between eastern and western populations but it is likely to involve the Nullarbor Plain and deserts north of this region. Other Australian passerine species also show a genetic or morphological discontinuity between eastern and western populations at this zone (Ford 1987; Joseph *et al.* 2002; Driskell and Christidis, unpublished data).

The lack of phylogeographic structure among the eastern Australian specimens (Figs 1, 3) could be explained by a period of recent population growth. All three samples show evidence consistent with population expansion (Table 2, Fig. 2). The current distribution of white-winged fairy-wrens indicates that it is patchily and sparsely distributed through the large central deserts (Blakers *et al.* 1984). During the glacial maxima of the Pleistocene, these central deserts expanded to cover a large expanse of the continent (Galloway and Kemp 1981; Miller *et al.* 1997). Presumably, the habitat of white-winged fairy-wrens – spinifex, heath, mallee, acacia scrub and open woodlands (Schodde 1982*b*; Blakers *et al.* 1984; Rowley and Russell 1977) – experienced a concomitant contraction during these periods. The idea that the ranges of birds that currently inhabit drier regions of the interior were compressed into fringing remnants during the Pleistocene has been proposed many times previously (Keast 1961; Ford 1974; Schodde 1982*a*). The genetic evidence that some populations of *M. leucopterus* have undergone recent range expansion is consistent with this Pleistocene refugium hypothesis.

If we assume that the mitochondrial genes in the white-winged fairy-wrens are evolving in a clock-like manner, we can apply a molecular substitution rate to our data to estimate the dates of divergence of the samples. An approximate rate of 2% sequence divergence per million years has been independently calibrated for a large number of avian orders (reviewed in Klicka and Zink 1997), although for passerines a rate of 2.4% sequence divergence per million years appears to be more accurate (Tarr and Fleischer 1993). Applying the rate of 2.4% to the Dirk Hartog–Western Australia split gives a date of divergence of 120 000 years ago. This divergence post-dates the date of isolation of both Dirk Hartog Island and Western Australian birds from conspecifics in eastern Australia (approximately 180 000–190 000 ago). The divergence between Barrow Island birds and all other sampled populations occurred earlier still, at approximately 220 000–245 000 ago.

All divergences are Late Pleistocene and although the isolation of the island populations presumably occurred through a rise in sea levels, the most recent rise in sea levels (to their present depth) approximately 8000 years ago is too recent to have been the isolating mechanism. Sea levels have been at or below 10 m below current levels for about 90% of the last 250000 years (Voris 2000). Both Dirk Hartog and Barrow Islands are within the same bathymetric contour of the coast and ocean depth between the islands and their nearest mainland connection is at or below 10 m (data from WORLDBATH; see Acknowledgments). From these data we would expect both islands to show a similar level of genetic differentiation from mainland populations and that this level would be lower than that which we have observed. However, even during the periods in which the mainland was connected to the island, the intervening areas may have been covered with sand dunes or other inhospitable habitat that acted as a barrier. A similar situation is proposed for the Pleistocene land bridges across Bass Strait (Galloway and Kemp 1981). In this case, the greater 'off-shore' distance of Barrow Island still adequately explains the greater level of genetic differentiation observed between its population of fairy-wrens and the mainland.

A number of questions about plumage evolution in white-winged fairy-wrens remain unanswered. First, if black plumage on Barrow and Dirk Hartog Island evolved independently, what factors have led to this convergence? Second, if there is gene flow between the population on Dirk Hartog Island and the mainland, how does this gene flow express itself in plumage variation and do hybrid individuals regularly occur either on the mainland or on Dirk Hartog Island? Lastly, how genetically distinct are the mainland western and eastern populations of *M. leucopterus leuconotus*? There are no obvious east–west morphological or plumage differences in this subspecies, but our genetic data suggest little or no gene flow between the west and east. These questions await further research.

### Acknowledgments

This research was supported by the National Science Foundation (grants IBN 9724053, DEB 9623526, and a Research Experience for Undergraduates supplement), the Norman Wettenhall Foundation, and Western Australian Petroleum (WAPET). All molecular work was conducted in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum. Les Christidis at the Museum of Victoria, Scott Edwards and Sievert Rohwer at the Burke Museum, and Ron Johnstone at the Western Australian Museum and Melanie Rathburn of Queen's University kindly provided specimens. Leo Joseph and Les Christidis provided helpful comments on the manuscript, thereby greatly improving it. Andy Kern assisted with the interpretation of statistical results. Information on the unpublished primers designed by Seutin and Bermingham and used in this study can be found at <http://nmg.si.edu/bermlab/htm>. The WORLDBATH database of bathymetric and topographic data is found at <http://ingrid.ldgo.columbia.edu/SOURCES/WORLDBATH/bath/>.

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**Appendix 1. Specimens of white-winged fairy-wrens examined in this study**

Specimens, abbreviations and collection localities included in study are shown. Figure label = label used in Fig. 1; Specimen No. = specimen or collection number; *M. l.* = *Malurus leucopterus*; W. Aust. Mus. = Western Australia Museum; Mus. Victoria = Museum Victoria; Bk. Cons. Pk = Brookfield Conservation Park, SA

Taxon	Figure label	Collection locality	Latitude	Longitude	Specimen No.	Source
<i>M. l. edouardi</i>	BIs1 1	Barrow I.	20°47'S	115°28'E	BW005	Pruett-Jones
<i>M. l. edouardi</i>	BIs1 2	Barrow I.	20°47'S	115°28'E	BW038	Pruett-Jones
<i>M. l. edouardi</i>	BIs1 3	Barrow I.	20°47'S	115°28'E	BW003	Pruett-Jones
<i>M. l. edouardi</i>	BIs1 4	Barrow I.	20°47'S	115°28'E	BW011	Pruett-Jones
<i>M. l. edouardi</i>	BIs1 5	Barrow I.	20°47'S	115°28'E	BW002	Pruett-Jones
<i>M. l. edouardi</i>	BIs1 6	Barrow I.	20°47'S	115°28'E	BW006	Pruett-Jones
<i>M. l. edouardi</i>	BIs1 7	Barrow I.	20°47'S	115°28'E	BW007	Pruett-Jones
<i>M. l. edouardi</i>	BIs1 8	Barrow I.	20°47'S	115°28'E	BW001	Pruett-Jones
<i>M. l. leucopterus</i>	DHIs1 1	Dirk Hartog I.	25°50'S	113°05'E	A26803	W. Aust. Mus.
<i>M. l. leucopterus</i>	DHIs1 2	Dirk Hartog I.	25°50'S	113°05'E	A26804	W. Aust. Mus.
<i>M. l. leucopterus</i>	DHIs1 3	Dirk Hartog I.	25°50'S	113°05'E	A26802	W. Aust. Mus.
<i>M. l. leucopterus</i>	DHIs1 4	Dirk Hartog I.	25°50'S	113°05'E	A26806	W. Aust. Mus.
<i>M. l. leucopterus</i>	DHIs1 5	Dirk Hartog I.	25°50'S	113°05'E	DH81	M. Rathburn
<i>M. l. leucopterus</i>	DHIs1 6	Dirk Hartog I.	25°50'S	113°05'E	DH60	M. Rathburn
<i>M. l. leucopterus</i>	DHIs1 7	Dirk Hartog I.	25°50'S	113°05'E	DH98	M. Rathburn
<i>M. l. leucopterus</i>	DHIs1 8	Dirk Hartog I.	25°50'S	113°05'E	DH141	M. Rathburn
<i>M. l. leucopterus</i>	DHIs1 9	Dirk Hartog I.	25°50'S	113°05'E	DH239	M. Rathburn
<i>M. l. leucopterus</i>	DHIs1 10	Dirk Hartog I.	25°50'S	113°05'E	DH309	M. Rathburn
<i>M. l. leuconotus</i>	WA 1	Wiluna	26°35'S	120°13'E	SVE1265	Burke Museum
<i>M. l. leuconotus</i>	WA 2	Wiluna	26°35'S	120°13'E	SVE1267	Burke Museum
<i>M. l. leuconotus</i>	WA 3	Cape Range	21°55'S	114°04'E	MV2512	Mus. Victoria
<i>M. l. leuconotus</i>	QLD 1	Bulloo Downs	28°32'S	142°57'E	D084	Mus. Victoria
<i>M. l. leuconotus</i>	QLD 2	Bulloo Downs	28°32'S	142°57'E	D075	Mus. Victoria
<i>M. l. leuconotus</i>	QLD 3	Bulloo Downs	28°32'S	142°57'E	D085	Mus. Victoria
<i>M. l. leuconotus</i>	QLD 4	Bulloo Downs	28°32'S	142°57'E	D086	Mus. Victoria
<i>M. l. leuconotus</i>	QLD 5	Bulloo Downs	28°32'S	142°47'E	D093	Mus. Victoria
<i>M. l. leuconotus</i>	NSW 1	Menindee	32°24'S	142°26'E	SAR7202	Burke Museum
<i>M. l. leuconotus</i>	NSW 2	Menindee	32°24'S	142°26'E	SAR7203	Burke Museum
<i>M. l. leuconotus</i>	NSW 3	Menindee	32°24'S	142°26'E	SAR7199	Burke Museum
<i>M. l. leuconotus</i>	NSW 4	Menindee	32°24'S	142°26'E	SAR7201	Burke Museum
<i>M. l. leuconotus</i>	SA 1	Bk. Cons. Pk	43°21'S	139°31'E	WW040	Pruett-Jones
<i>M. l. leuconotus</i>	SA 2	Bk. Cons. Pk	43°21'S	139°31'E	WW014	Pruett-Jones
<i>M. l. leuconotus</i>	SA 3	Bk. Cons. Pk	43°21'S	139°31'E	WW030	Pruett-Jones
<i>M. l. leuconotus</i>	SA 4	Bk. Cons. Pk	43°21'S	139°31'E	WW020	Pruett-Jones
<i>Malurus lamberti</i>		Bk. Cons. Pk	43°21'S	139°31'E	VW104	Pruett-Jones
<i>Malurus melanocephalus</i>		Atherton, Qld	17°16'S	145°28'E	RBW01	J. Karubian