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PHYLOGEOGRAPHY OF MITOCHONDRIAL DNA IN TWO SPECIES OF WHITE-EYES IN AUSTRALIA

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ABSTRACT.—Variation was assessed among mitochondrial DNAs (mtDNAs) from geographically dispersed individuals of two species of white-eyes (*Zosterops lutea* and *Z. lateralis*) in Australia. The survey revealed high levels of intraspecific divergence. The mtDNA of *Z. lutea* was paraphyletic; eastern samples were more closely related to eastern *Z. lateralis* than to western *Z. lutea*. The mtDNA of *Z. lateralis* showed a major phylogenetic break within the distribution of one subspecies, *Z. l. halmaturina*, and was relatively uniform across four subspecies distributed along the east coast of Australia. The discordance between species boundaries and the distribution of mtDNA haplotypes is probably due to historical hybridization. The poor correspondence between subspecies boundaries and mtDNA discontinuities in *Z. lateralis* implies that patterns of morphological variation may reflect local processes more than evolutionary history. In a sample of the morphologically divergent island race, *Z. l. chlorocephala*, mtDNA haplotypes were identical to those found on the adjacent mainland. However, the frequencies of mtDNA variants differed considerably between the two places. These data suggest that the island race is recently derived from the mainland, but that current gene flow is rare. *Received 1 July 1991, accepted 23 February 1992.*

STUDIES OF geographic variation in birds have contributed significantly to the development of evolutionary theory, particularly concerning speciation (Mayr 1963) and adaptation (James 1970). The evolutionary significance of geographic variation depends upon the extent to which that variation has a genetic basis and reflects the joint effects of selection and history. In the past, this has been difficult to assess in birds because studies of genetic variation, relying upon the identification of polymorphic enzyme loci, have usually reported low levels of intraspecific variation (Barrowclough 1983, Zink and Remsen 1986). Recently, analysis of mtDNA has revealed a more sensitive source of genetic variation, for reasons that are now well documented (Avise 1986, Moritz et al. 1987), and is becoming popular in studies of geographic genetic variation.

To date, a small number of studies have assessed intraspecific variation of mtDNA in widespread passerines, with contrasting results. A continentwide survey of the Red-winged Blackbird (*Agelaius phoeniceus*), which shows considerable geographic variation in morphology, revealed a large number of mtDNA haplotypes characterized by low levels of sequence divergence and widespread geographic distribution (Ball et al. 1988). A similar widespread distribution of mtDNA clones was documented for the Song Sparrow (*Melospiza melodia*), a species that exhibits relatively little morphological variation in the regions surveyed (Zink 1991). In contrast, mtDNA variation in some species is characterized by distinct geographic structuring (e.g. *Branta canadensis*, Shields and Wilson 1987, Van Wagner and Baker 1990; *Ammodramus maritimus*, Avise and Nelson 1989; *Pomatostomus temporalis*, Edwards and Wilson 1990; *Colaptes auratus*, Moore et al. 1991; and *Passerella iliaca*, Zink 1991). In some of these cases (e.g. *Ammodramus maritimus*), the geographic pattern of mtDNA variation is discordant with that of morphological variation. mtDNA also has provided diagnostic markers between avian sibling species, although genetic distance between congeners is usually small (summarized in Avise and Zink 1988). Phylogenetic analysis of mtDNA and allozymes from congeneric sparrows (*Ammodramus*, Zink and Avise 1990) revealed affinities other than those predicted from morphological comparisons.

It is apparent, therefore, that observed morphological variation, which has so often been used to interpret evolutionary relationships in birds, may not always reflect evolutionary history. In this paper, we report on mtDNA vari-

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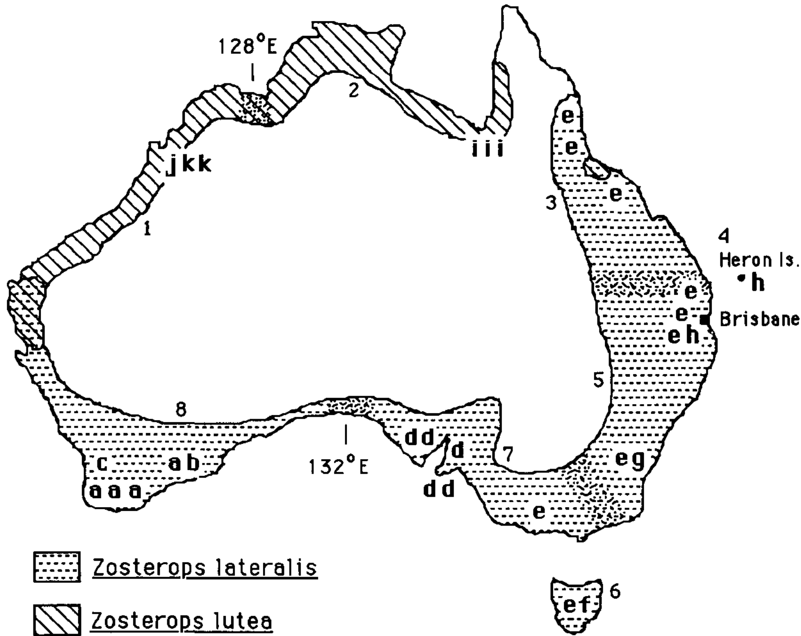

Fig. 1. Map of Australia showing distribution of *Zosterops lutea* (**1**, *Z. l. balstoni*; **2**, *Z. l. lutea*) and *Z. lateralis* (**3**, *Z. l. ramsayi*; **4**, *Z. l. chlorocephala*; **5**, *Z. l. familiaris*; **6**, *Z. l. lateralis*; **7**, *Z. l. halmaturina*; **8**, *Z. l. gouldi*). Stippled areas indicate subspecies boundaries where appropriate. Lowercase letters are positioned to indicate sampling localities, and each represents mtDNA clone type of a single individual.

ation within two species of white-eyes (*Zosteropidae*) that exist, for the most part, allopatrically in Australia—the Silvereye (*Zosterops lateralis*) and the Yellow White-eye (*Z. lutea*). The species and subspecies of the Indo-Australian *Zosterops* have been determined by plumage color and morphometrics (Mees 1969). It is now recognized that difficulties arise because of the immense color variation within some species and because of potentially convergent similarities between some species (Moreau 1957).

Zosterops lateralis, the Silvereye, is a very mobile and wide-ranging species found throughout most of eastern, southern and southwestern Australia, where six subspecies are recognized (Fig. 1; Blakers et al. 1984). Winter flocks on the southern and central east coast comprise both local residents and migrants (Lane 1966) from more southerly (including Tasmania) populations (Kikkawa 1968). Of special concern to the present work is *Z. l. chlorocephala*, a race that maintains high-density populations on small wooded cays of the southern Great Barrier Reef. Mees (1969) concluded that the race is of recent origin and, yet, it is morphologically distinct from the mainland race and is the most abundant land bird on some of the cays, such as Heron Island (Kikkawa 1970). Similarities in plumage color of island birds and those on the adjacent mainland coast suggest that *chlorocephala* was derived from a nearby mainland population (Mees 1969).

Zosterops lutea, the Yellow White-eye, is endemic to the north coastal parts of Australia and arguably comprises two subspecies (Fig. 1; Mees 1961, Blakers et al. 1984). There is an outlying population on the east coast of the continent, within the range of the Silvereye (Lavery and Grimes 1974) and, in the west, the range of the Yellow White-eye overlaps narrowly with that of the Silvereye. A third species, *Z. citrinella*, occurs only on wooded islands off the far northeastern tip of the continent (Blakers et al. 1984) and was not considered in this study.

The work presented here is part of a broader study of genetic variation in the Heron Island population of *Z. l. chlorocephala*, which is also the subject of a detailed study in evolutionary ecology (e.g. Kikkawa et al. 1986, Catterall et al. 1989). Here we focus on macrogeographic patterns of variation in the Silvereye, using samples of the Yellow White-eye as outgroups. The specific questions addressed are: (1) is the mtDNA variation geographically structured; (2)

are the evolutionary lineages revealed by mtDNA analysis concordant with species and subspecies boundaries; and (3) does mtDNA provide information on the origin and divergence of the Heron Island race?

MATERIALS AND METHODS

For restriction-enzyme site mapping of mtDNA, *Z. lateralis* specimens from Brisbane ($n = 2$) and Heron Island ($n = 1$) were collected during the breeding season of 1989. Dissected heart tissues were either snap frozen and stored at −80°C (Heron Island specimen) or processed immediately (Brisbane specimens). For macrogeographic analysis, additional samples of heart from *Z. lateralis* ($n = 21$) and *Z. lutea* ($n = 6$) from several localities around Australia (Fig. 1) were kindly donated by R. Schodde (CSIRO, Canberra) and L. Christidis (Museum of Victoria). Subspecific identifications of all specimens were based on morphology and collection location. Voucher specimens are listed in the Appendix.

The mtDNA was purified by ultracentrifugation in a cesium chloride gradient, followed by dialysis, as described in Dowling et al. (1990). For restriction-enzyme site mapping, the Brisbane and Heron Island mtDNAs were digested to completion with 16 five- or six-base-pair-recognizing restriction endonucleases (listed in Fig. 2) in both single- and double-digestion combinations. After digestion, mtDNA fragments were end-labelled with ^{32}P-nucleotides and their sizes determined by electrophoresis through agarose and polyacrylamide gels, followed by autoradiography. Fragment sizes were calculated against *Ava*I/*Bgl*II-digested lambda-phage DNA as a size standard.

The positions of all restriction sites for 15 of the enzymes were mapped relative to each other by double digestion (Dowling et al. 1990). Sites for *Spe*I could not be mapped because of the presence of several small fragments that had no internal sites for any of the other mapped enzymes. The construction of a restriction-enzyme site map tests the interpretation of fragment changes as site gains or losses, and allowed for an alignment of the Silvereye mtDNA molecule with that of previously characterized and sequenced mtDNAs. It also allowed for an assessment of the distribution of restriction sites, particularly polymorphic sites, within the mitochondrial genome. If the sites were strongly clustered, estimates of sequence divergence may be biased because of variation in evolutionary rates among genes (Brown 1985).

Sequence divergences between mtDNAs were estimated from comparisons of restriction-enzyme sites using the methods of Nei and Tajima (1983) in a program described in Nei et al. (1985). This matrix of divergences was used to construct a dendrogram by UPGMA clustering (Sneath and Sokal 1973). Restriction-enzyme site character data (presence/absence) were analyzed using both Dollo-parsimony and Wagner-parsimony criteria (DeBry and Slade 1985, Swofford and Olsen 1990) using PAUP (version 3.0, supplied by D. L. Swofford, Illinois Natural History Survey, Champaign). The stability of the resultant parsimony cladograms was evaluated by bootstrapping (Felsenstein 1985) using PAUP.

The mtDNA variation among an additional 15 individuals from Heron Island and an additional 15 individuals from the adjacent mainland (Brisbane) was nondestructively assayed for three endonucleases (*Eco*RV, *Ava*I and *Hin*dIII) that in combination diagnose the known east-coast variants of *Z. lateralis* mtDNA. This was done by Southern hybridization analysis using purified ^{32}P-labelled Silvereye mtDNA as a probe against total genomic DNA extracted from a small volume of blood. Hybridization procedures were based on those described by Church and Gilbert (1984) and were performed at 65°C with a final high-stringency wash in 0.2× SSC at 65°C. Composite results from these three enzymes were used to designate mtDNA haplotypes to the 15 Heron Island and the 15 Brisbane individuals. The two populations were treated as demes and the amount of among-deme genetic variation (G_{ST}) was estimated as described in Takahata and Palumbi (1985), using a program provided by Stephen Palumbi (Univ. Hawaii, Honolulu). The observed G_{ST} was tested against a null hypothesis of random genetic variation across demes, using the bootstrap procedure described by Palumbi and Wilson (1990).

RESULTS

A restriction-enzyme site map for Silvereye mtDNA is presented in Figure 2. The total size of the mtDNA molecule of this species is estimated to be approximately 17.5 kilobases, in the upper-middle of the size range (16.2 to 18.1 kb) so far reported for birds (Shields and Helm-Bychowski 1988). There were no cases of mtDNA length variation or heteroplasmy. The map for the Heron Island individual was identical to that of one of the Brisbane individuals (clone h; see below). Three restriction-enzyme sites that appear to be widely conserved across the animal kingdom (see Carr et al. 1987) are indicated on the map. These sites were used to align and orientate the restriction-enzyme site map of the Silvereye to the mtDNA sequence of the chicken (*Gallus gallus*; Desjardins and Morais 1990), allowing for the identification of particular regions such as the rRNA genes and the control region. The sites assayed in *Z. lateralis* appear to be fairly evenly distributed along the length of the molecule, although the subset of polymorphic sites tend to be less uniformly distributed (Fig. 2). No polymorphic sites were

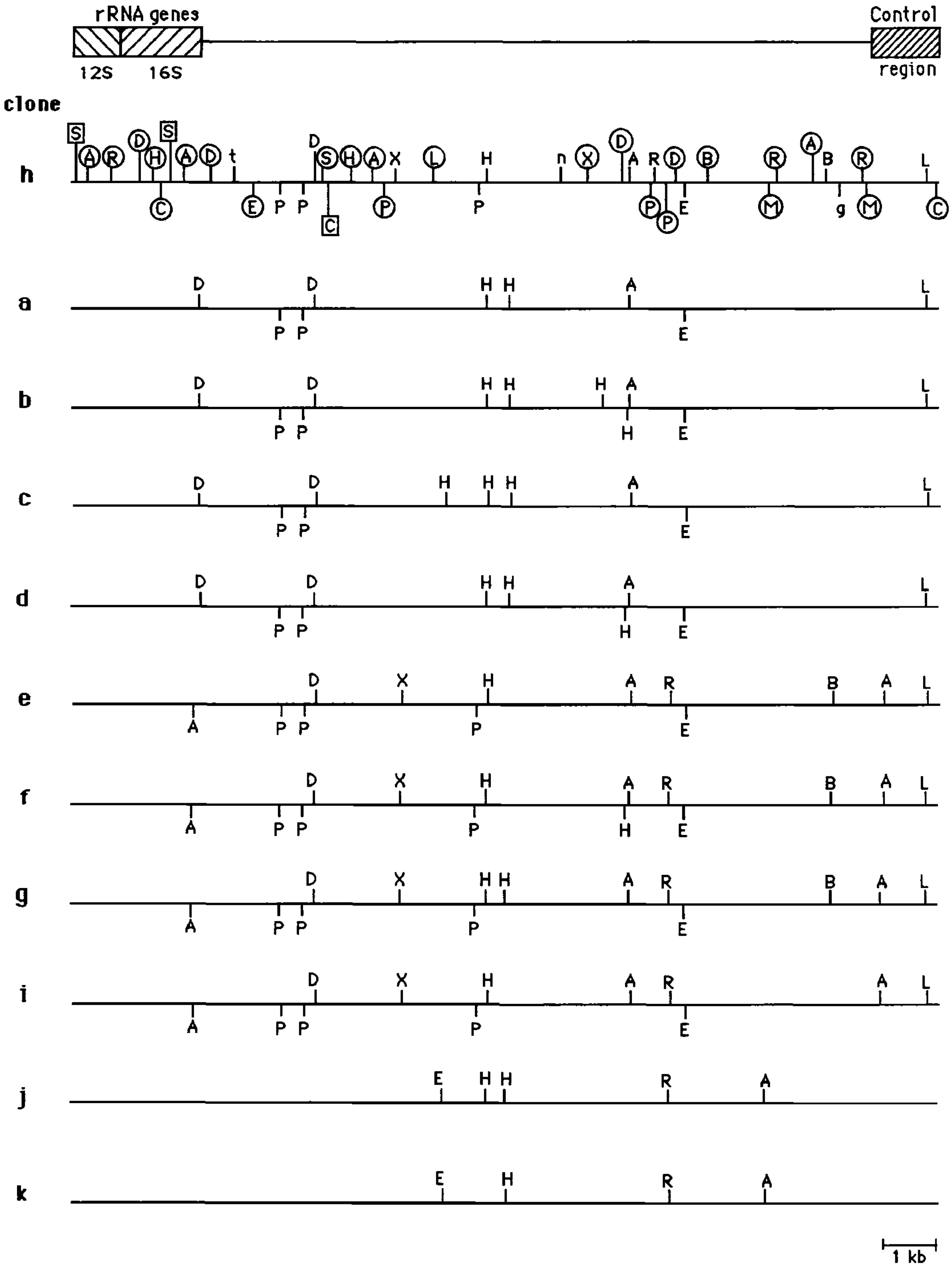

Fig. 2. The mtDNA restriction-enzyme site maps for *Zosterops lateralis* (clones a–h) and *Z. lutea* (clones i–k). Sites enclosed by boxes represent sites conserved across animal kingdom and used to align map to chicken mitochondrial genome (top of figure). Other sites that were monomorphic among all *Zosterops* sampled are circled. Enzymes designated as follows: A = *Ava*I; B = *Bam*HI; D = *Dra*I; E = *Eco*RI; R = *Eco*RV; H = *Hin*dIII; C = *Hpa*I; M = *Mlu*I; P = *Pvu*II; S = *Sac*II; L = *Sal*I; X = *Xba*I; g = *Bgl*II; t = *Bst*EII; n = *Nco*I. *Spe*I fragments were not mapped. Enzymes designated by lowercase letters not used to screen individuals in phylogeographic analysis.

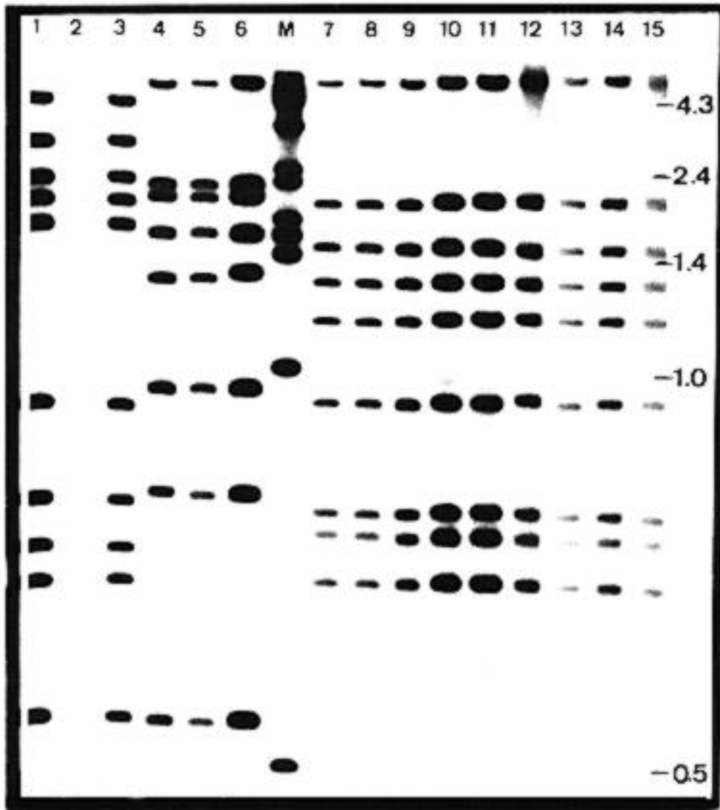

Fig. 3. Autoradiograph of a 1.0% agarose gel following electrophoretic separation of fragments of *Zosterops* mtDNA digested with *Spe*I. Fragment sizes (kb) of size standard (*Ava*I/*Bgl*II digested lambda phage DNA, lane M) shown at right. Three distinct mtDNA clones can be seen: *Z. lutea* (Western Australia), lanes 1–3; *Z. lateralis* (Tasmania and Victoria), lanes 4–6; and *Z. lateralis* (South Australia and Western Australia), lanes 7–15.

mapped within the ribosomal genes; two (for *Ava*I and *Sal*I) were mapped within the control region.

Among the 30 individuals analyzed for 13 restriction enzymes, 62 different restriction-enzyme sites were observed, with an average of 39 sites (representing 234 nucleotides or 1.3% of the mtDNA genome) scored per individual. Of the 62 sites observed, 28 were polymorphic; 16 varied among the 24 individuals of *Z. lateralis*, while 22 varied among the six individuals of *Z. lutea*. Of the 13 enzymes used to screen all 30 individuals, 10 enzymes produced two or more fragment profiles, as illustrated by *Spe*I (Fig. 3). Fragment sizes for these polymorphic enzymes are available from the senior author upon request. The sizes of fragments (in base pairs) produced by the remaining three (monomorphic) enzymes are as follows: *Hpa*I (12,000, 3,500, and 1,700), *Mlu*I (15,400 and 1,800), and *Sac*II (12,300, 3,200, and 1,700). Sizes of fragments greater than about 8,000 bp are approximate only.

Eleven different mtDNA clones were represented among the 30 individuals (Table 1). Within *Z. lateralis*, eight different clones were observed, the most common (clone e) being represented by eight individuals spanning eastern Australia (subspecies *ramsayi*, *familiaris*, *halmaturina* and *lateralis*; Fig. 1). The next most common clones, d and a, were localized to southern (*halmaturina*, $n = 5$) and to southwestern (*gouldi*, $n = 4$) Australia, respectively. Clone h, found only in subspecies *familiaris* ($n = 1$) on the mainland, was also identified on Heron Island (*chlorocephala*, $n = 1$). The remaining four clones were each represented by a single individual from various sites around the southern part of

TABLE 1. Restriction-endonuclease descriptions and distributions of mtDNA haplotypes among white-eyes *Zosterops lateralis* and *Z. lutea*. Uppercase letters in descriptions, from left to right, correspond to restriction-fragment profiles produced by digestion with endonucleases *Ava*I, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Hpa*I, *Mlu*I, *Pvu*II, *Sac*II, *Sal*I, *Spe*I, and *Xba*I. Restriction profiles designated by adjacent letters of alphabet differ by only a single restriction-enzyme site; nonadjacent letters denote a difference of two or more restriction-enzyme sites (following Avise and Nelson 1989). By combining data from all enzymes, each individual was assigned a composite mtDNA haplotype (designated by a lowercase letter).

mtDNA haplotype	mtDNA description	Subspecies designation[a]	No. birds
a	CADAABAACAAAA	*gouldi*	4
b	CADAAFAACAAAA	*gouldi*	1
c	CADAAHAACAAAA	*gouldi*	1
d	CADAAEAACAAAA	*halmaturina*	5
e	EBCABCAADAAEB	*ramsayi, familiaris, halmaturina, lateralis*	9
f	EBCABDAADAAEB	*lateralis*	1
g	EBCABBAADAAEB	*familiaris*	1
h	CBCABCAADAAEB	*familiaris, chlorocephala*	2
i	EACABCAADAAFB	*lutea*	3
j	AAACBBAAAABCA	*balstoni*	1
k	AAACBAAAAABCA	*balstoni*	2
		Total	30

[a] Subspecies represented by haplotypes a–h belong to *Z. lateralis*, and i–k to *Z. lutea*.

the continent. Within *Z. lutea*, three clones were recognized: clone i in the east (*lutea*, $n = 3$), and clones j (*balstoni*, $n = 1$) and k (*balstoni*, $n = 2$) in the west (Fig. 1). Clearly, the distribution of mtDNA haplotypes in these two species bears a strong relationship to geography.

Estimates of percent sequence divergence between clones ranged from 0.17% to 4.91% (Table 2). The mean percent sequence divergence among the 11 clones was 2.24%. The mean among *Z. lateralis* clones was 1.48% and among those of *Z. lutea* was 3.12%; for both species, this divergence was largely explained by the differences between eastern and western mtDNA clones. The UPGMA dendrogram of these sequence-divergence data clearly separates eastern and western clones of *Z. lateralis*, at a level of approximately 2.3% divergence (Fig. 4). The east-west dichotomy within *Z. lateralis* occurred well within the range of *Z. l. halmaturina* rather than between *Z. l. halmaturina* and *Z. l. gouldi* as might be expected. Even more striking is the divergence of approximately 3.9% between eastern and western clones of *Z. lutea*, and also between western *Z. lutea* and all clones of *Z. lateralis* (Fig. 4).

For phylogenetic analyses, using the branch-and-bound algorithm of PAUP, presence or ab-

TABLE 2. Estimates of percent sequence divergence (lower left) between mtDNA types of *Zosterops lateralis* and *Z. lutea*. Estimates calculated from numbers of shared restriction-enzyme sites, presented in upper right. Number of restriction-enzyme sites used in each comparison on diagonal.

mtDNA type	mtDNA type: a	b	c	d	e	f	g	h	i	j	k
a	**46**	46	46	46	42	42	43	42	42	38	37
b	0.35	**48**	46	46	42	42	43	42	42	38	37
c	0.18	0.53	**47**	46	42	42	43	42	42	38	37
d	0.18	0.53	0.36	**47**	42	42	43	42	42	38	37
e	2.23	2.57	2.40	2.40	**50**	50	50	47	49	36	35
f	2.40	2.74	2.57	2.57	0.17	**51**	50	48	49	36	35
g	2.01	2.35	2.18	2.18	0.17	0.33	**51**	48	49	37	36
h	1.97	2.23	2.05	2.05	0.69	0.51	0.51	**48**	47	36	35
i	2.23	2.57	2.40	2.40	0.34	0.50	0.50	0.69	**50**	36	35
j	2.82	3.18	3.00	3.00	4.44	4.62	4.16	4.09	4.44	**44**	43
k	3.08	3.45	3.26	3.26	4.74	4.91	4.44	4.37	4.74	0.19	**43**

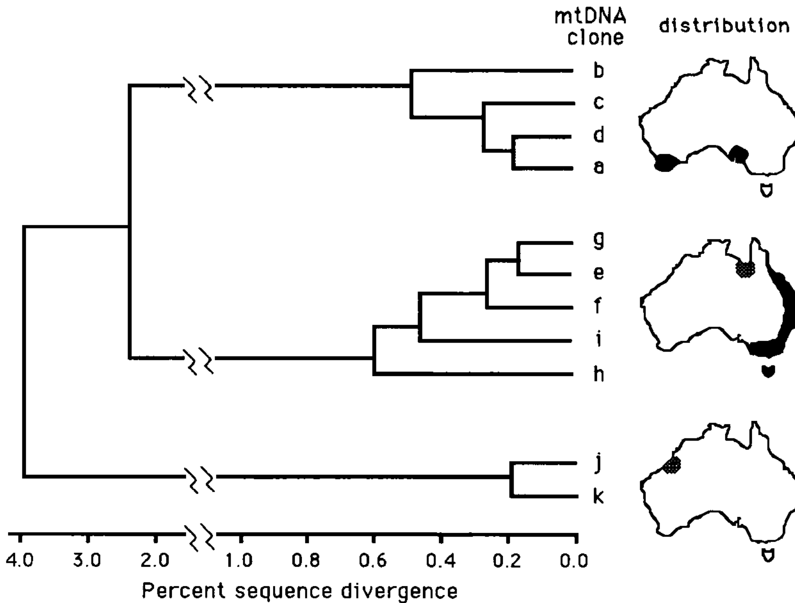

Fig. 4. UPGMA dendrogram based on sequence-divergence estimates. Distributions of *Z. lateralis* mtDNA clones are solid black, while those for *Z. lutea* are gray.

sence of restriction sites were treated as character states. Initial analyses, using all three clones of *Z. lutea* as outgroups, precluded monophyly of the ingroup because of the unexpected affinity of eastern *Z. lutea* clones with eastern *Z. lateralis* clones. These analyses were rejected and, subsequently, only the two western clones of *Z. lutea* (clones j and k) were designated as outgroups. The consensus Dollo-parsimony cladogram, generated by 100 bootstrap replications with a branch-and-bound search, revealed that the eastern Australian samples of *Z. lateralis* (clones e–h) and *Z. lutea* (clone i) form a strongly supported clade (Fig. 5). The western samples of *Z. lateralis* (clones a–d) that formed a tight group in the UPGMA analysis (Fig. 4) are not united by any derived character states (cf. Fig. 2) and, therefore, are unresolved in the phylogenetic analysis.

The mtDNA clone (clone h) found in the island race (*Z. l. chlorocephala*) and in one sample from Brisbane (*Z. l. familiaris*) was the sister group to other east-coast clones in the phylogenetic analysis (Fig. 5) and was also the most distinctive of these (Fig. 4). To further investigate the distribution of mtDNA variants between the Heron Island population and those on the mainland (specifically Brisbane), larger samples (each of $n = 15$) were assayed with *Ava*I, *Hin*dIII, and *Eco*RV by Southern hybridization. The composite haplotypes (Table 3) showed a major difference in frequencies of mtDNA clones between the two locations. In nine of the samples from Brisbane, digestion with *Eco*RV revealed a new fragment pattern, differing from others by a site gain. The sequence divergence between the two populations, corrected for within population variation, is 1.5%. The corresponding values within populations were 0.18% for Heron Island and 0.48% for Brisbane.

The difference in frequencies of mtDNA clones between the two populations is highly significant (heterogeneity $X^2 = 22.8$, $P < 0.005$). The G_{ST} value, obtained by treating the two populations as demes, is 0.30. The maximum value obtained in 100 randomizations was only 0.08, indicating that the value of 0.30 represents significant among-population differentiation (cf. Palumbi and Wilson 1990).

DISCUSSION

Phylogeography of mtDNA variation in Z. lateralis.—The clearest result within *Z. lateralis* is the separation of an east-coast group from other samples, suggesting a long-term separation of eastern and western populations. The sequence divergence of 2.3% between these two phylogeographic groups is among the highest values reported within a species of bird and is well

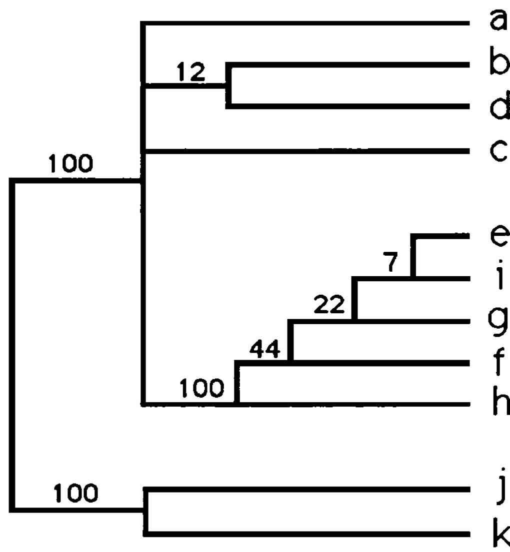

Fig. 5. Relationships between mtDNA clones as described by a Dollo 50%-majority-rule consensus tree, generated by 100 bootstrap replications of branch-and-bound search by PAUP program. Tree length was 38 steps, and consistency index was 0.737. Percentages of bootstrap replications that supported each node are given.

TABLE 3. Occurrence of composite mtDNA haplotypes among Brisbane (n = 15) and Heron Island (n = 15) populations of *Zosterops lateralis*. Uppercase letters in descriptions, from left to right, correspond to restriction-fragment profiles produced by digestion with endonucleases *Eco*RV, *Ava*I, and *Hin*dIII. Letter designations for each endonuclease follow those in Table 1, except that haplotype C for *Eco*RV represents a new haplotype not observed previously.

mtDNA type	Heron Island		Brisbane	
	n	Percent	n	Percent
BCC	14	93	1	7
BEC	1	7	5	33
CEC	0	0	9	60

within the range of previously reported interspecific divergences (e.g. Avise and Zink 1988, Shields and Helm-Bychowski 1988, Avise and Nelson 1989, Tegelstrom et al. 1990, Van Wagner and Baker 1990, Zink and Dittmann 1991, Zink et al. 1991). In comparison, a continent-wide survey of mtDNA diversity in the Red-winged Blackbird revealed very little diversity or geographic structuring, with a maximum observed sequence divergence of only 0.8% (Ball et al. 1988). Even in the Seaside Sparrow, for which two geographically defined clusters of mtDNA clones were observed, maximum sequence divergence was estimated to be only 1.0% (Avise and Nelson 1989).

The geographic location of the break between the two major assemblages of mtDNA from *Z. lateralis* occurs to the east of the "Eyrean" and "Nullarbor" biogeographical barriers, discontinuities defined by species-level comparisons in other Australian passerines (Keast 1961, Kikkawa and Pearse 1969, Cracraft 1986, Ford 1987). However, the mtDNA break does correspond to the "Mallee" barrier, recognized as possibly the most severe southern barrier for semiarid bird species during the peak of the last glaciation, and as a region that now has numerous contact zones (Ford 1987). It would be of interest to survey mtDNA from other widespread species to evaluate the congruence between intraspecific and interspecific biogeographic breaks.

Comparison with patterns of morphological variation.—The relationships and evolutionary history implied by the mtDNA analysis are incongruent with species and subspecies boundaries. Most strikingly, mtDNA from *Z. lutea* is paraphyletic with respect to *Z. lateralis*, since the eastern mtDNA clones of *Z. lutea* are more closely related to those of eastern *Z. lateralis* than they are to western clones of *Z. lutea*. On morphological grounds, Mees (1961) treated *Z. lutea* as an independent species, rather than as part of a wide-ranging superspecies, and suggested that it probably evolved in northwestern Australia, derived from rainforest inhabitants of the Lesser Sundas, Indonesia. If so, the species would have extended its range towards the eastern portion of the continent, where populations may now be isolated from the west by the "Carpentarian" geomorphological barrier commonly recognized by zoogeographers (Macdonald 1969, Ford 1987; barrier "B" in fig. 9 of Cracraft 1986). The presence of the Carpentarian barrier was also reflected in the geographic pattern of length and site variation in mtDNA of Grey-crowned Babblers (*Pomatostomus temporalis*; Edwards and Wilson 1990).

Previous studies reporting discordance between mtDNA haplotypes and species classifications have attributed this to either the sorting of ancestral polymorphisms (Neigel and Avise 1985; e.g. Avise et al. 1990), or to introgressive hybridization (Ferris et al. 1983, Tegelstrom 1986). The former hypothesis seems an unlikely

explanation in the present case because of the large sequence divergences involved. Introgressive hybridization seems more plausible; even a low level of hybridization may be sufficient to establish a neutral mtDNA clone within a foreign population (Takahata and Slatkin 1984). On the east coast, *Z. lutea* has established a population within the range of *Z. lateralis* (Fig. 1; Lavery and Grimes 1974), providing the opportunity for hybridization between the two species. However, the two species are now ecologically and morphologically distinct, and no hybrids have been reported. It would be of interest to sample individuals of *Z. lutea* from current areas of overlap (e.g. the west and northeast coasts; Fig. 1) to determine whether the presumed hybridization was an isolated incident. If hybridization was recent or is continuing, it should be evident in nuclear DNA polymorphisms (Degnan in prep.).

The distribution of mtDNA variation is also discordant with the currently recognized subspecies boundaries (Fig. 1). The existence of an mtDNA clone (clone e) common to the entire east coast and extending across the range of four subspecies is consistent with high levels of historical gene flow. This gene flow probably continues, since migrating individuals from the southern part of the continent are known to mix with resident individuals further north, at least during the nonbreeding season (Lane 1966, Kikkawa 1968). It seems plausible that not all individuals complete the return journey before settling to breed, particularly in view of the occasional record of movement of banded birds in a continued northerly direction at the end of the migratory season (Lane 1966). A particular anomaly exists in the southern part of mainland Australia, where the recognized boundary of the two subspecies, *gouldi* in the west and *halmaturina* in the east (Mees 1969), lies much further to the west of the continent than does the mtDNA discontinuity. The race *gouldi* is conspicuous in lacking the grey back that is characteristic of all other silvereye races, and Mees (1969) considered it a well-marked subspecies that "has clearly lived in isolation for a long time." However, both Mees (1969) and Ford (1987) recognized that, currently, a narrow zone of intergradation exists between these two southern subspecies.

Lack of correlation between the distributions of *Z. lateralis* subspecies and the patterns of mtDNA variation indicate that morphology alone may not provide a clear picture of the evolutionary history of this species. Not only are historical barriers and morphological boundaries discordant, but also the magnitude of mtDNA divergence and morphological difference are unlinked. The greatest difference in mtDNA occurs between individuals of *Z. l. gouldi* (and western *Z. l. halmaturina*) and eastern *Z. l. halmaturina*, subspecies that differ in plumage color and only slightly in morphometrics (Mees 1961). Conversely, the mtDNA difference between the much larger island race, *Z. l. chlorocephala*, and mainland birds is relatively small.

External morphology, so often used to define taxonomic groups and to make evolutionary inferences in birds, can show substantial variation in the context of very little or no genetic differentiation. A similar situation was reported for the Seaside Sparrow (Avise and Nelson 1989), for which mtDNA data suggested that the subspecific taxonomy did not adequately reflect the evolutionary genetic relationships of the populations sampled. As suggested by Zink and Avise (1990) in reference to similar observations in sparrows (*Ammodramus*), morphological variation, to the extent it is heritable, may be more a reflection of local selection pressures than evolutionary history.

However, remember that mtDNA represents only a single molecular lineage. In *Z. lateralis*, mtDNA phylogeographies are consistent with type I of Avise et al. (1987), although these authors proposed that type IV may prove to be the most common in birds that are highly mobile. If this observed spatial separation of mtDNA clones, with distinct phylogenetic discontinuities, is due to long-term, extrinsic barriers to gene flow, then analysis of RFLPs for nuclear DNA (Degnan in prep.) should reveal congruent patterns of geographic variation.

Origin and divergence of Heron Island population.—The mtDNA of the Heron Island population (subspecies *chlorocephala*) is closely related to that of the adjacent mainland populations, including *Z. l. familiaris* from Brisbane. Identical mtDNA haplotypes were found in the two populations, strongly suggesting that the Heron Island population may have derived from the nearby eastern Australian mainland (although it is also possible that the immediate ancestors were from other island races to the east; J. Kikkawa pers. comm.). However, the significant frequency differences of composite haplotypes derived from three restriction-enzyme frag-

ment patterns suggest that the level of gene flow from the mainland to the island is presently very low. This is consistent with the observation that occasional winter immigrants of the mainland race disappear by the onset of the breeding season on Heron Island, so that no interbreeding with island birds is known (Kikkawa 1970).

The low mtDNA differentiation between the two populations is of particular interest in view of the substantial morphological differences that exist between them. A low sequence divergence indicates a short separation time so that morphological differentiation must have occurred rapidly, presumably as the result of strong selection. In addition, the continued existence of at least two founding mtDNA lineages in the island population also suggests a relatively short separation time (or occasional gene flow), since the random extinction of mtDNA lineages can be very rapid under the kind of demographic conditions that may prevail in an island population (Avise et al. 1984).

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APPENDIX. All specimens used in analysis. Registered specimens housed as voucher skins in the CSIRO collection, Canberra, Australia, except for B17363 which is in Museum of Victoria, Australia. Unregistered specimens designated by MV series field numbers also housed in Museum of Victoria. For remaining three specimens, skins were not retained.

Field number	Species	Subspecies	Collection locality	Museum no.	mtDNA clone
C926	*lateralis*	*ramsayi*	Helenvale, QLD	39879	e
F105	*lateralis*	*ramsayi*	Clarke Range, QLD	41408	e
F267	*lateralis*	*ramsayi*	Atherton, QLD	41564	e
H293	*lateralis*	*chlorocephala*	Heron Island, QLD	Unregistered	h
C401	*lateralis*	*familiaris*	Agnes Water, QLD	39353	e
C452	*lateralis*	*familiaris*	Kroombit Tops, QLD	39404	e
B37	*lateralis*	*familiaris*	Brisbane, QLD	Unregistered	h
B38	*lateralis*	*familiaris*	Brisbane, QLD	Unregistered	e
S3078	*lateralis*	*familiaris*	Gunghalin, ACT	38457	g
S3079	*lateralis*	*familiaris*	Gunghalin, ACT	38458	e
MV068	*lateralis*	*familaris*	Grampians, VIC	B17363	e
B724	*lateralis*	*lateralis*	Upper Blessington, TAS	38901	e
B725	*lateralis*	*lateralis*	Upper Blessington, TAS	38902	f
D357	*lateralis*	*halmaturina*	Sinclairs Gap, SA	40332	d
D381	*lateralis*	*halmaturina*	Sinclairs Gap, SA	40356	d
D388	*lateralis*	*halmaturina*	Port Pirie, SA	40363	d
42487	*lateralis*	*halmaturina*	Kangaroo Island, SA	42487	d
42568	*lateralis*	*halmaturina*	Kangaroo Island, SA	42568	d
MV191	*lateralis*	*gouldi*	Esperance, WA	Unregistered	a
MV192	*lateralis*	*gouldi*	Esperance, WA	Unregistered	b
MV240	*lateralis*	*gouldi*	Albany, WA	Unregistered	a
MV241	*lateralis*	*gouldi*	Albany, WA	Unregistered	a
MV265	*lateralis*	*gouldi*	Pemberton, WA	Unregistered	a
MV305	*lateralis*	*gouldi*	Hoffmans Hill, WA	Unregistered	c
F353	*lutea*	*lutea*	Normanton, QLD	41649	i
F354	*lutea*	*lutea*	Normanton, QLD	41650	i
F362	*lutea*	*lutea*	Kurumba, QLD	41658	i
D058	*lutea*	*balstoni*	Point Torment, WA	39181	j
D059	*lutea*	*balstoni*	Point Torment, WA	39182	k
D060	*lutea*	*balstoni*	Point Torment, WA	39183	k