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Phylogenetic Relationships between Oviparous and Viviparous Populations of an Australian Lizard (*Lerista bougainvillii,* Scincidae)

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Viviparity has evolved from oviparity in many vertebrate lineages, and species that contain both oviparous and viviparous populations offer the best opportunity for a detailed examination of the processes involved in this major life-history transition. However, although several such species have been reported, none have been the subject of detailed phylogenetic analyses. We examine such a case within the Australian scincid lizard *Lerista bougainvillii.* Data were obtained by sequencing a 314-bp segment of mitochondrial cytochrome *b* from 32 individuals from 17 populations of *L. bougainvillii* and two morphologically similar congeneric species (*L. dorsalis* and *L. microtus*). Sequences were aligned and analyzed using parsimony and distance methods. The resultant matriarchal phylogeny resolved the populations of *L. bougainvillii* into three major groups: a population from NSW; a group predominantly from Eyre Peninsula; and a less well-defined group from the central part of the species range. The NSW and Eyre Peninsula groups are oviparous and are quite divergent from other *L. bougainvillii* populations and from each other. The central group contains both viviparous and oviparous populations, and seems to represent a more recent radiation within the species. Our results indicate that viviparity has evolved at least twice within the genus *Lerista,* because the viviparous *L. microtus* is not closely related to viviparous populations of *L. bougainvillii.* The lack of phylogenetic separation of mtDNAs from viviparous and oviparous populations within *L. bougainvillii* relative to strong geographic structure within the latter indicates that populations with different reproductive modes are indeed conspecific. *Lerista bougainvillii* is thus the first vertebrate species for which intraspecific bimodality in reproductive mode can be claimed with any certainty.

INTRODUCTION

The evolution of viviparity ("live-bearing") involves a profound shift—the elimination of the independent egg stage from the life cycle. Accordingly, the causes and consequences of this phylogenetic transition have been the focus of much scientific speculation. Many selective forces have been hypothesized to play a role in stimulating the evolutionary transition from oviparity to viviparity (Tinkle and Gibbons, 1977; Shine, 1985). Attempts to test such alternative hypotheses on the evolution of viviparity must ultimately rely on comparisons between oviparous and viviparous taxa, but widespread phylogenetic conservatism in reproductive modes means that such comparisons are often confounded by phylogenetic differences between the groups. Ideally, comparative research should be conducted with organisms that differ only in the characteristics being studied (e.g., Harvey and Pagel, 1991). Squamate reptiles offer by far the best opportunity to make such comparisons, with at least 98 origins of viviparity compared to less than 20 in all other vertebrates combined (Blackburn, 1981; Shine, 1985). Some reptilian groups are particularly valuable in this respect: for example, viviparity has evolved at least 11 times among Australian skinks (Shine, 1985). The taxa that offer the greatest opportunity for unconfounded comparisons are reproductively bimodal species: that is, those that have been reported to include both oviparous and viviparous populations.

Unfortunately, many of the reportedly reproductively bimodal "species" have proven to be composites of genetically distinct oviparous and viviparous species when subjected to further taxonomic analysis (Shine, 1985). However, there are two well-studied reptile "species" which are currently thought to be bimodal in their reproduction, as follows:

(i) The European lizard *Lacerta vivipara* (Lacertidae) is viviparous throughout most of its range, but oviparous in the extreme southwest portion (Heulin *et al.*, 1989). However, although lizards from the oviparous and viviparous populations are similar in many respects, they differ in allele frequencies (Bea *et al.*, 1990) and produce significant numbers of embryonic malformations when hybridized in the laboratory (Heulin *et al.*, 1989). Thus, Heulin *et al.* (1989, p. 342) concluded that "more research is needed before we can say

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whether or not the oviparous and viviparous races of *Lacerta vivipara* really belong to the same species."

(ii) The other well-studied "species" reported to exhibit both modes of reproduction is the Australian scincid lizard *Lerista bougainvillii*, the focus of our study. Most *Lerista* species are thought to be oviparous, but viviparity has been reported in *L. microtis* and in some populations of *L. bougainvillii* (Greer, 1989). Females in most oviparous populations of *L. bougainvillii* lay fully shelled eggs, containing embryos that are relatively undeveloped (at stages 31 to 34 following the classification system of Defaure and Hubert, 1961). This is similar to the situation in other oviparous skinks (Shine, 1985). The viviparous populations give birth to fully developed offspring at stage 40 of the Defaure and Hubert classification, and these offspring are enclosed only by transparent uncalcified membranes (Rawlinson, 1974; Qualls *et al.*, 1995). Morphological and electrophoretic analyses suggest that the oviparous and viviparous forms of *L. bougainvillii* are indeed conspecific (e.g., they show no fixed allelic differences in liver proteins at any of the loci tested; Qualls *et al.*, 1995), but the crucial evidence to evaluate this possibility (a phylogenetic analysis of oviparous and viviparous populations) has not been available until the current study.

Lerista bougainvillii occur in the southeastern Australian mainland and on southern offshore islands, and the mode of reproduction is related to location. With one possible exception (Gippsland; see Greer, 1989; Qualls *et al.*, 1995), all mainland populations are oviparous. The viviparous *L. bougainvillii* occur only in isolated populations on islands off the southern coast of Australia (Kangaroo Island, Tasmania, and Bass Strait islands such as Flinders and Chappell Island; Fig. 1). The live-bearing population on Kangaroo Island is

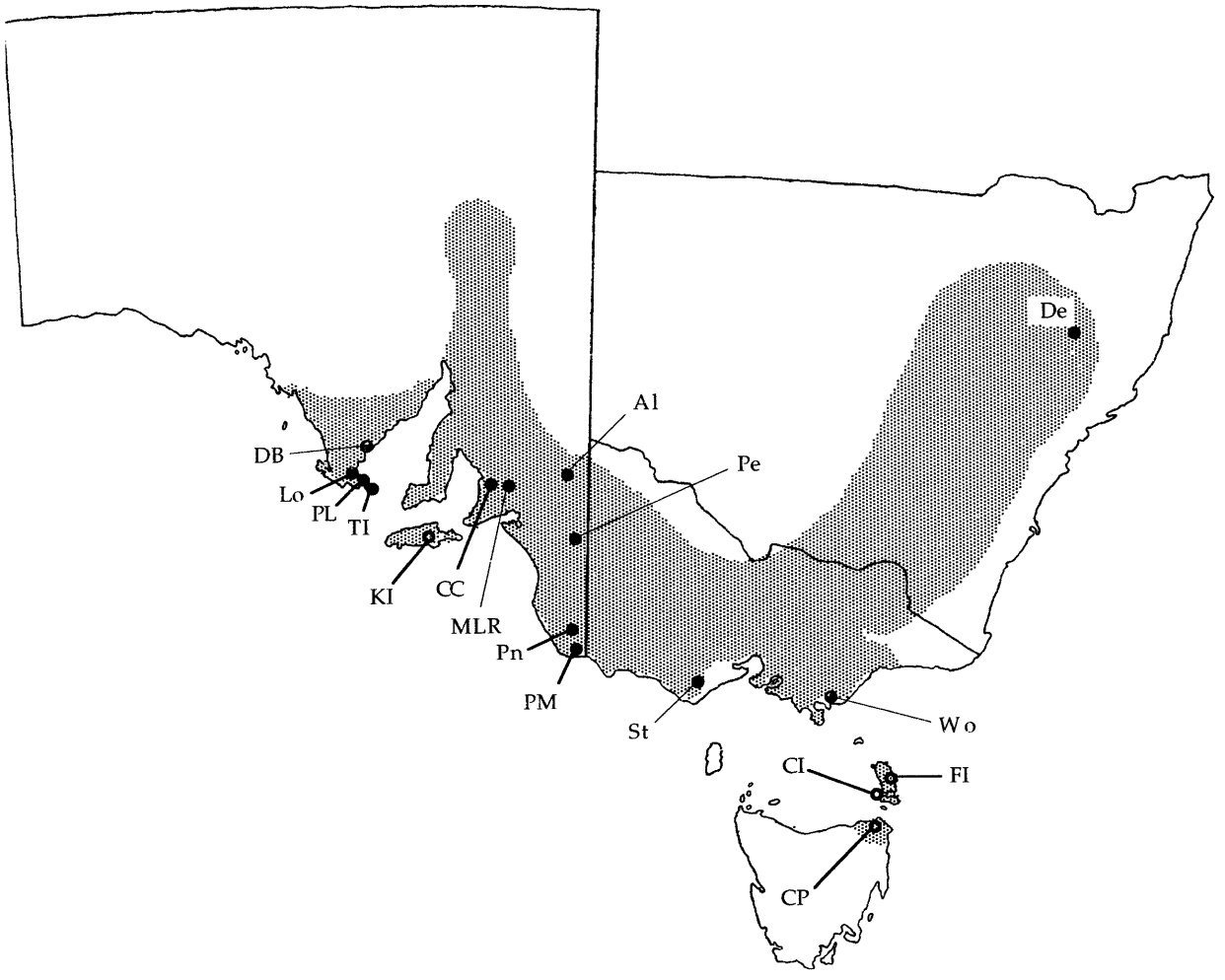

FIG. 1. Geographical locations of the 17 *L. bougainvillii* populations used. Mainland egg-layers: Denman (De), Woodside (Wo), Stonyford (St), Port MacDonnell (PM), Penola (Pn), Peebinga (Pe), Alawoona (Al), Mount Lofty Ranges (MLR), Cudlee Creek (CC), Louth (Lo), Taylors Island (TI), Port Lincoln (PL), Dutton Bay (DB). Island live-bearers: Flinders Island (FI), Chappell Island (CI), Cape Portland (CP), Kangaroo Island (KI). The shaded area represents the geographic distribution of the species. Dots represent oviparity, and circles represent viviparity (from Qualls *et al.*, 1995).

separated from the other live-bearing populations (Tasmania and Bass Strait islands) by approximately 1000 km, raising the possibility of not one but two separate origins of viviparity within *L. bougainvillii* on these colder offshore islands.

Although recent work has examined intraspecific phylogenetic relationships of *L. bougainvillii* using evidence from morphology and allozymes (Qualls *et al.,* 1995), these analyses were entirely phenetic, and the low level of geographic variation in both morphological and electrophoretic characters means that some uncertainty remains about phylogeographic patterns within this taxon. The high level of variation and strong geographic orientation of mitochondrial DNA (mtDNA) sequences make it an ideal molecule for investigating evolutionary relationships within species (Avise *et al.,* 1987; Avise, 1991; Moritz *et al.,* 1987). Here we present an analysis of a 314-bp mtDNA sequence to test for evolutionary divergence among live-bearing and egg-laying populations of *Lerista bougainvillii.*

MATERIALS AND METHODS

Samples of frozen liver or muscle tissue from 26 specimens of *L. bougainvillii* were obtained from the South Australian Museum (SAM). These specimens were collected from the following locations: NSW, two from Denman; SA, two from Cudlee Creek, one from Penola, one from Port Macdonnell, three from Alawoona, one from Port Lincoln, one from Dutton Bay, four from Taylor's Islet, one from Peebinga, one from Mt. Lofty Ranges, two from Kangaroo Island; Tasmania, two from Chappell Island, one from Flinders Island, and two from Cape Portland. For six Victorian specimens, DNA was extracted from frozen cell lysate sent from SAM, three from Stonyford, and three from Woodside. DNA was also extracted from muscle tissue from two fresh SA specimens collected by C. Qualls, one from Louth (99) and one from Kangaroo Island (20). In total, we used 32 individuals from 17 populations (four islands and 13 mainland populations). Two outgroups were used, *Lerista dorsalis* and the viviparous *L. microtus,* on the basis of their morphological resemblance to *L. bougainvillii.* Collection locations of the 17 populations of *L. bougainvillii* are shown in Fig. 1, SAM accession numbers are provided in Table 1.

The tissue samples were kept on dry ice at all times and stored at −70°C. Small samples of the tissue were ground in a 2-ml microfuge tube using liquid nitrogen and a mortar. DNA was extracted from frozen tissue grindate using a chelex-based extraction protocol (Singer-Sam *et al.,* 1989), following the procedures of Walsh *et al.* (1991) and Moritz *et al.* (1992a). A tiny amount of tissue homogenate (<5 mg) was boiled for 7 min in 1 ml of chelex suspension, which consisted of Chelex-100 biotechnology grade chelating resin (Bio-Rad) in 10 mM Tris, 0.1 mM EDTA, pH 7.6 ($TE_{0.1}$ buffer). The DNA/chelex extraction solutions were centrifuged and stored at 5°C. The chelex DNA preparations were used as a template for the double-stranded polymerase chain reaction (PCR) amplification using primers that span 426 bp of the mitochondrial cytochrome *b* gene (cyt *b*). The two oligonucleotide primers used were MVZ04 (5′-GCA GCC CCT CAG AAT GAT ATT TGT CCT C-3′), a 28-mer derived from the heavy strand of cyt *b;* and MVZ05 (5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′) is a 28-mer light strand primer (Smith and Patton, 1991; Smith *et al.,* 1992).

Twenty microliters of the chelex DNA preparations was used in 100 µl of PCR solution containing 1× buffer, 2.0 mM $MgCl_2$, 200 µM of each dNTP, 1 µM of each primer, 0.02 units/µl of *Thermus acquaticus* (Taq) DNA polymerase (Boehringer Mannheim), and capped with 100 µl of mineral oil. A negative control tube containing 20 µl of sterile water instead of template was included with each PCR run of 10 cycles (94°C for 1 min, 50°C for 1 min, 72°C for 1 min) and 30 cycles (94°C for 30 s, 50°C for 30 s, 72°C for 1 min) and 5 min extension at 72°C. The DNA was precipitated out of solution and resuspended in 40 µl of 10 mM $TE_{0.1}$ (pH 7.6) and then purified by running each 40-µl sample on a 4% nondenaturing acrylamide gel (Accugel). The bands were visualized under UV light and the band corresponding to the correct size was removed. DNA was extracted from the bands by soaking twice in 200 µl of 10 mM $TE_{0.1}$ (pH 7.6) at 37°C. The DNA was precipitated out of solution, resuspended in 30 µl of $TE_{0.1}$, and stored at −20°C. The final yield of DNA was between 20 and 100 ng/µl in each 30-µl tube.

DNA sequencing was performed using the Sanger method of dideoxy-mediated chain termination (Sanger *et al.,* 1977). The oligonucleotide MVZ04 was used to sequence the PCR products directly using Sequenase (Version 2.0, United States Biochemical) following the protocol of Cassanova *et al.* (1990). The entire 369-bp segment between the primers was sequenced for all 34 individuals. The amount of DNA solution used was adjusted so that the ratio of primer to template was approximately 20:1. All samples produced readable sequences. Sequencing using the oligonucleotide MVZ05 to verify the sequences resulted in double sequences and "ghost" bands, probably due to mispriming. A primer internal to MVZ05 was designed for sequencing, by comparing the sequences obtained using MVZ04. The 3′ end of the primer was located at position 34 on the original sequences (position 14,216 in *Mus;* Bibb *et al.,* 1981) and was 20 nucleotides long (CAC CAT CAA ACA TTT CAG CC). For all 34 individuals using this primer, 314 bp of the 369-bp sequences were verified.

Sequences of both strands were obtained from autoradiographs and aligned by eye. The distribution and amount of variation among the sequences were analyzed manually using the MacClade program Version 3.01 for Macintosh PCs (Maddison and Maddison,

1992). A computer program was written to analyze the number of transitions and transversions at each codon by pairwise comparisons of sequences. These were plotted against maximum-likelihood (ML) estimates of divergence using the ML option of DNAdist in PHYLIP Version 3.5c for Macintosh PCs (Felsenstein, 1993). Nonlinearity in the plots indicates increasing saturation of substitutions for that class of characters (Moritz *et al.*, 1992a).

Parsimony and distance methods were used to estimate phylogenetic relationships among sequences (Swofford and Olsen, 1990). Nucleotide sequences were analyzed by the maximum-parsimony (MP) method using PAUP Version 3.1.1 (Swofford, 1993). Each consistency index (CI) is based on sites that are informative under the conditions of parsimony (those sites with at least two different bases, each occurring in more than one taxon). Heuristic searches were conducted with 100 replicates of random addition of branches using only informative characters. The resolving power of the tree was tested by Bootstrap analysis using PAUP 3.1.1 with 100 replicates. Alternative phylogenetic hypotheses were tested using the Constraints option in PAUP. The resulting trees were then compared for differences in length (number of steps) and for the number of sites supporting each respective topology using MacClade Version 3.01 (Maddison and Maddison, 1992). Pairwise sequence distances were calculated using the DNAdist program of PHYLIP using Kimura's (1980) two-parameter method. The pairwise distances were analyzed by the neighbor-joining method (Saitou and Nei, 1987) using PHYLIP. Bootstrap pseudoreplicate distance matrices were constructed from 100 resamplings of the original alignment using DNAboot in PHYLIP. Neighbor-joining trees were constructed from each matrix and the results summarized as a majority rule consensus tree using the Consense program in PHYLIP.

TABLE 1

Collection Locations, South Australian Museum (SAM) Accession Numbers and GenBank Accession Numbers for the Specimens Used in Our Study: Thirty-two Individuals of *L. bougainvillii*, and Single Specimens Representing the Two Outgroups *L. dorsalis* and *L. Microtus*

Specimen	Collection location	SAM access number	Genbank access number
Wo1.egg	Woodside, Vic.	NMV D62024	AF020003
Wo2.egg	Woodside, Vic.	NMV D62025	AF020004
Wo3.egg	Woodside, Vic.	NMV D62026	AF020005
St1.egg	Stonyford, Vic.	V61	AF020006
St2.egg	Stonyford, Vic.	NMV D60956	AF020007
St3.egg	Stonyford, Vic.	NMV D60957	AF020008
PM.egg	Port Macdonnell, SA	W93 R34689	AF020009
Pn.egg	Penola, SA	H865 R32955	AF020010
Pe.egg	Peebinga, SA	R36682	AF020011
Al1.egg	Alawoona, SA	R37508	AF020012
Al2.egg	Alawoona, SA	R37509	AF020013
Al3.egg	Alawoona, SA	R37510	AF020014
CC1.egg	Cudlee Creek, SA	H433	AF020015
CC2.egg	Cudlee Creek, SA	H437	AF020016
Lo.egg	Louth, Vic.	N/A	AF020017
TI1.egg	Taylor's Islet, SA	LB1 R31140	AF020018
TI2.egg	Taylor's Islet, SA	LB2 R31141	AF020019
TI3.egg	Taylor's Islet, SA	LB3 R31142	AF020020
TI4.egg	Taylor's Islet, SA	LB4 R31143	AF020021
PL.egg	Port Lincoln, SA	PL70	AF020022
DB.egg	Dutton Bay, SA	R074 R32850	AF020023
MLR.egg	Mt Lofty Ranges, SA	R31583	AF020024
FI.live	Flinders Island, Tas.	R37946	AF020025
CI1.live	Chappell Island, Tas.	R37947	AF020026
CI2.live	Chappell Island, Tas.	R37948	AF020027
CP1.live	Cape Portland, Tas.	R37506	AF020028
CP2.live	Cape Portland, Tas.	R37507	AF020029
KI1.live	Kangaroo Island, SA	NP2399	AF020030
KI2.live	Kangaroo Island, SA	NP2325	AF020031
KI3.live	Kangaroo Island, SA	N/A	AF020032
De1.egg	Denman, NSW	R37513	AF020033
De2.egg	Denman, NSW	R37514	AF020034
dorsalis	—	NP2326	AF020035
microtus	Esperance, WA	R29496	AF020036

RESULTS

Sequences 314 bp in length read from both strands of DNA were obtained for 32 individuals of *L. bougainvillii* and the two outgroups. No deletions or additions were observed and the sequences were translated in a single open reading frame, suggesting that they were not derived from any pseudogene. The Genbank accession numbers for the DNA sequences are provided in Table 1. The magnitude of variation was high. Including both *L. bougainvillii* and outgroups, 109 (34%) of 314 nucleotides and 23 (22%) of 104 amino acids varied. Among the 109 variable nucleotides 23 (21%) were in the first codon position, 5 (5%) were in the second, and 81 (74%) were in the third codon position. Of these 109 variable sites, 82 (75%) varied in the ingroup. Nonetheless, the proportion of variable nucleotides within each of the eight populations for which there was more than one individual was low: Denman (0.6%, $n = 2$); Cudlee Creek (0.3%, $n = 2$); Alawoona (1.6%, $n = 3$); Stonyford (1.6%, $n = 3$); Woodside (0.6%, $n = 3$); Taylors Island (1.0%, $n = 4$); Kangaroo Island (2%, $n = 3$); and Cape Portland (0.6%; $n = 2$).

Transitions, especially between C and T (68% of all transitions), outnumbered transversions. Using pairwise comparisons of all the data, an average transition to transversion ratio of 2.6:1 was found with the outgroups included in the analysis, and a ratio of 3.03:1 for the ingroup alone. To test for saturation of base substitution (i.e., "multiple hits"), the numbers of transitions and transversions at each codon position from pairwise comparisons of sequences were plotted against ML estimates of divergence. A 3:1 transition to transversion ratio was used to calculate the ML distances.

Increases in the number of transitions at the third codon position were nonlinear relative to divergence estimates, indicating increasing saturation of substitutions for that class of characters. This nonlinearity was particularly obvious for comparisons between *L. bougainvillii* and the outgroups. Although third base transversions were linearly related to divergence estimates, first and second base position transversions were scattered relative to ML distance (only 6 and 23% of the variation in the first and second position can be explained by ML distance via linear regression, respectively).

Of the 109 variable sites, 76 were informative under the conditions of maximum parsimony. These informative sites were used in phylogenetic reconstructions using PAUP with equal weights. A heuristic search of this data set resulted in 540 equally parsimonious trees of 175 steps. A strict consensus tree was constructed (Fig. 2). All branches defining major geographic clusters were present in 100% of the 540 trees (CI = 0.805). The large number of equally parsimonious trees stems from the instability of the branching order of individuals within populations or between geographically close

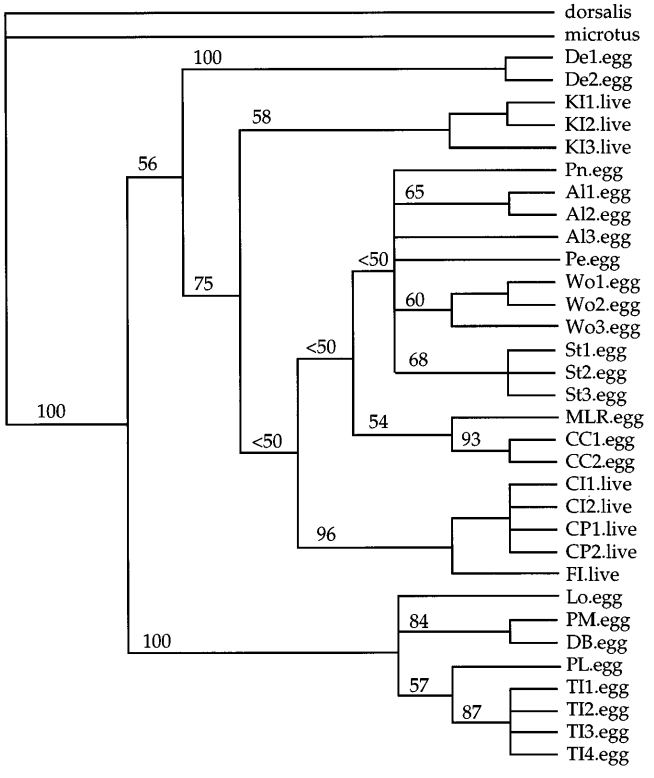

FIG. 2. A strict consensus tree generated from a heuristic search on informative characters using PAUP. Resolved branches represent 100% consensus. Each individual lizard tested is represented by a code that incorporates information on its geographic location (see caption to Fig. 1) and mode of reproduction ("egg," oviparity; "live," viviparity). The numbers indicate the percentage to which each branch was supported among the 100 pseudoreplicates derived from Bootstrap analysis.

populations. Although random addition of branches was used, it was possible that the heuristic search had become trapped in a local optimum (Page, 1993). To test this possibility of more parsimonious topologies between populations, a Branch and Bound search was used on a data set in which individuals within each population were collapsed into 17 population consensus sequences using IUPAC/IUB ambiguity codes. The consensus had the same topology as the MP tree for the full data set. The above analyses of transitions and transversions indicated that the third base codon position transitions were saturated among the more divergent sequences. However, searches conducted on a data set which excluded the third base of each codon did not differ from the MP tree in the topology of the basal branches.

All individuals from the same population formed monophyletic groups in this analysis and many populations from similar geographic regions consistently grouped together with strong support from pseudoreplication (Fig. 2). In the basal branch, populations from Eyre Peninsula (Dutton Bay, Louth, Taylors Island, and Port Lincoln) formed a strongly supported monophyletic lineage, together with a lizard from Port Macdonnell in eastern South Australia. Bass Strait populations (Flinders Island, Chappell Island, and Tasmania) also formed a monophyletic group. Populations from Mount Lofty Ranges and Cudlee Creek in South Australia (henceforth referred to as the Fleurieu Peninsula group) branched off earlier than a group of phylogenetically unresolved populations from Woodside and Stonyford in Victoria and populations from Peebinga, Alawoona, and Penola in South Australia.

This phylogenetic hypothesis generated by MP was tested to see if it differed from those generated by *a priori* predictions based on a monophyletic origin of viviparity within the genus *Lerista* and within *L. bougainvillii.* To test the first possibility, a search was conducted with all viviparous individuals, including *L. microtus,* constrained to form a monophyletic group. The analysis revealed 1100 equally parsimonious trees of 197 steps, 22 steps more than the most parsimonious tree (winning sites test; $\chi^2 = 22$, $df = 1$, $P < 0.001$). This result indicates that viviparity evolved separately within the two species.

In the above trees, viviparous Kangaroo Island populations and viviparous Bass Strait populations did not form a monophyletic group, suggesting a diphyletic origin of viviparity within *L. bougainvillii.* However, the branches separating the live-bearing clades are poorly supported (<50%) in pseudo-replications (Fig. 2). To test the possibility of a monophyletic origin of viviparity within *L. bougainvillii,* we conducted a search with all viviparous individuals of *L. bougainvillii* constrained to form a monophyletic group. The analysis revealed 1094 equally parsimonious trees of 177 steps, only two steps more than the most parsimonious tree

implying diphyly within *L. bougainvillii* ($\chi^2 = 2$, $df = 1$; $P = 0.18$, using Fisher's exact test).

Phenetic analysis revealed similar results. Pairwise distances for all individuals of *L. bougainvillii* and the outgroups were calculated. The observed sequence differences ranged from 0 to 15% within *L. bougainvillii*, whereas distances between *L. bougainvillii* and the outgroups ranged from 19 to 24%. The resultant neighbor joining tree (Fig. 3) showed that individuals clustered into their respective populations, as with the MP tree. The grouping of population "clades" is in agreement with MP analysis, notably the Eyre Peninsula, Bass Strait, and Fleurieu Peninsula groups. However, the two groups of live-bearers were now monophyletic, although with weak support from pseudo-replication (Fig. 3).

DISCUSSION

The pattern of nucleotide substitution for cyt *b* sequences has been well documented (Brown *et al.*, 1982; Brown, 1983, 1985; Wilson *et al.*, 1985; Meyer *et al.*, 1990; Meyer and Wilson, 1990; Smith and Patton, 1991; Hedges *et al.*, 1991; Irwin *et al.*, 1991; Moritz *et al.*, 1992a). Our results for *L. bougainvillii* conform to these patterns: synonymous differences are more numerous than nonsynonymous differences, variation is highest in the third base position of codons, and transitions exceed transversions. Although a transition bias is evident in most of the ingroup comparisons, this bias (3:1) is lower than found in studies on some other species (Moritz *et al.*, 1992b; Hedges *et al.*, 1991; Arevalo *et al.*, 1994). This lower ratio was probably due to a saturation of transitions at the third base position of codons. As expected, most of the nucleotide variation occurred in this third base position, which is effectively redundant in mtDNA. The level of first base variation was also high (21%), but comparable to that seen in previous studies (Moritz *et al.*, 1992b).

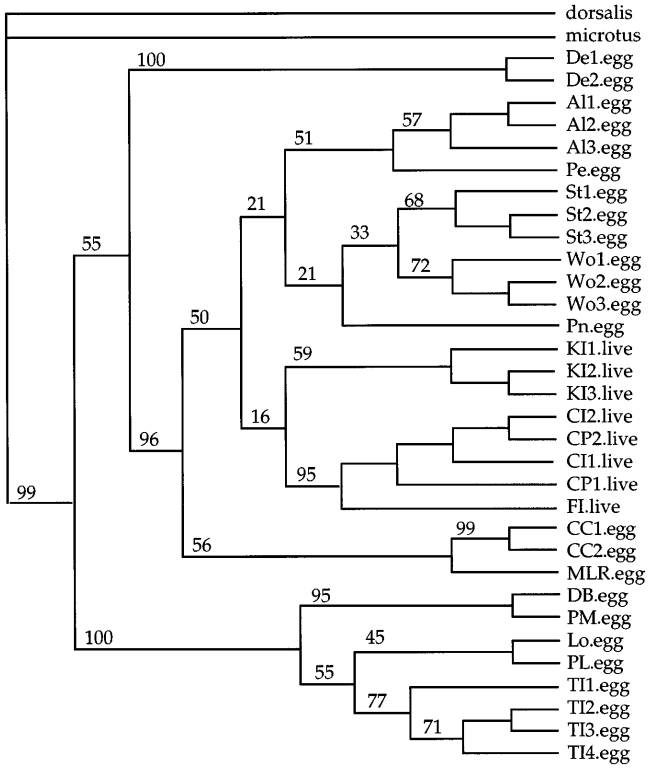

FIG. 3. Neighbor-joined tree derived from pairwise distances. The numbers indicate the percentage to which each branch was supported among the 100 pseudoreplicates derived from Bootstrap analysis. See caption to Fig. 1 for further explanation.

The amount of variation within *L. bougainvillii* and outgroups was high for both nucleotides (36%) and amino acids (22%). However, variation among individuals from the same population was minimal (between 0.3 and 2%), indicating that the mtDNA within a population is fairly homogeneous. Based on pairwise comparisons, the variation among individuals of *L. bougainvillii* was between 0 and 14%, and hence is comparable to levels of variation found between species in previous studies (Hedges *et al.*, 1991; Moritz *et al.*, 1992a).

Figure 4 shows the best supported features of the intraspecific phylogeny of *L. bougainvillii* based on our data. The strong tendency for all individuals within the same population to cluster together in the analyses, and for neighboring populations to cluster together (Figs. 2 and 3), indicates that there is underlying phylogeographic structure. The only exception to this clustering was the single Port Macdonnell specimen that grouped with lizards from the Eyre Peninsula over 500 km away. There are three possible explanations for this anomaly. First, the Port Macdonnell and Eyre Peninsula populations may represent the descendants of a mtDNA variant that occurred in the ancestral population. The amount of variation found between disjunct populations in this study makes this possibility unlikely, as gene flow would be needed to maintain the homogeneity of mtDNA haplotypes. The second possibility is that the similarity of the Port Macdonnell mtDNA to that of Eyre Peninsula could be due to a subsequent invasion or hybridization. This hypothesis could be tested by investigating populations along the coast between these "disjunct" populations. The final possibility is that there was a mislabeling of specimens. More individuals from this population and of other populations along the coast need to be tested.

The different methods used for phylogenetic reconstruction resulted in trees that generally agreed in their major branching sequences. The resultant phylogenetic hypothesis groups the 32 individuals into clades corresponding to six geographical areas: NSW, SA and Victoria, Kangaroo Island, Bass Strait, Fleurieu Peninsula, and Eyre Peninsula. Of these groups two (Eyre Peninsula and NSW) differ substantially from each other and from the rest of the *L. bougainvillii* popula-

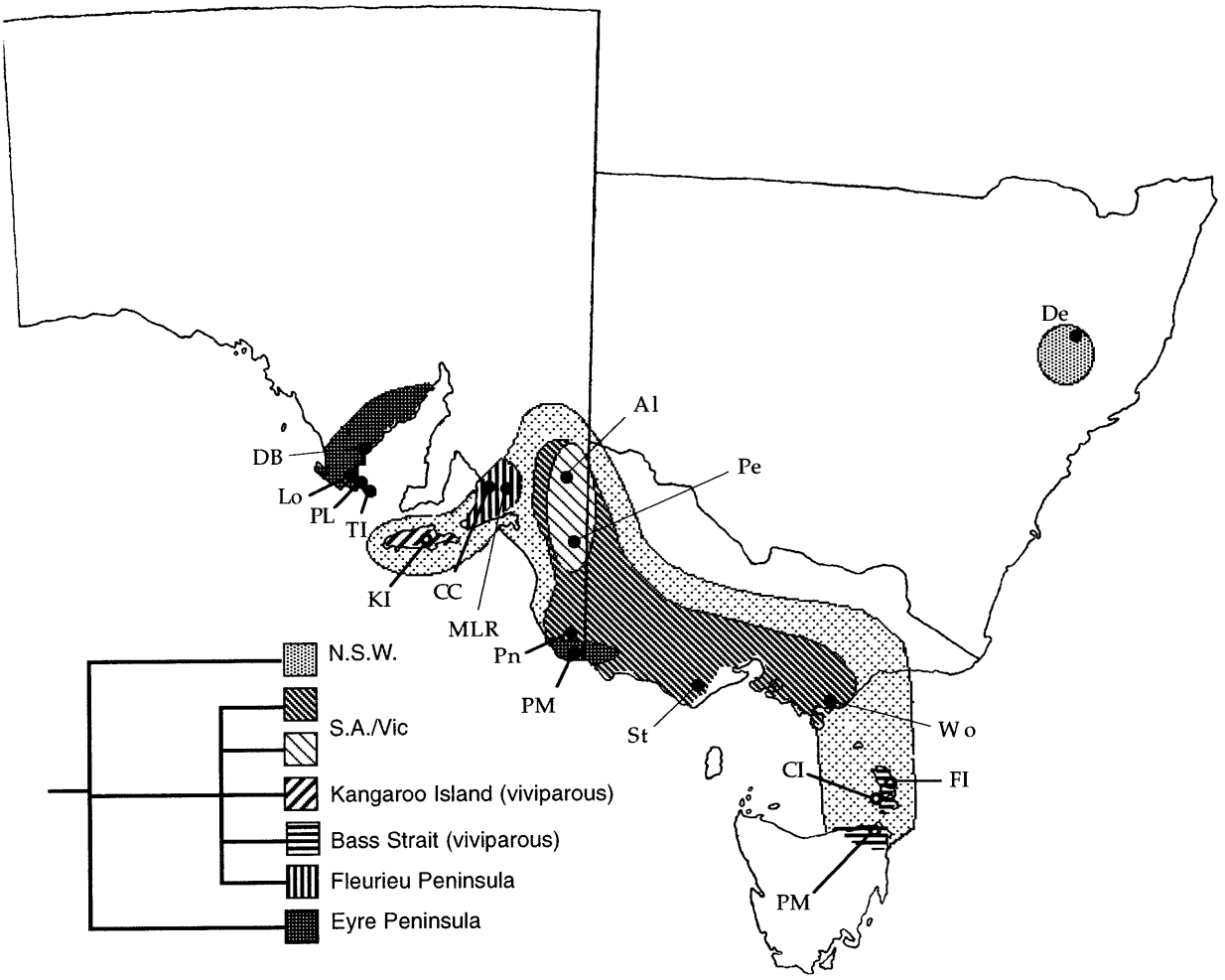

FIG. 4. Phylogeographic relationships of populations of *L. bougainvillii,* with viviparous populations indicated. See caption to Fig. 1 for listing of localities.

tions. These populations form the extreme north east (NSW) and west (Eyre Peninsula) of the species range and are likely to represent early divergences of now-isolated populations. The genetic distinctiveness of the Eyre Peninsula lizards from their Fleurieu Peninsula neighbors suggests the existence of some barrier to gene flow, perhaps the Gawler and Flinders Ranges. This division roughly parallels patterns of intraspecific phylogeography in some bird taxa (Degnan and Moritz, 1992), suggesting that a general vicariance event may be responsible (see also Heatwole, 1987).

The remaining lineage includes lizards from the Fleurieu Peninsula, South Australian and Victorian populations south of the Murray river, together with the viviparous island populations. We were unable to determine the phylogenetic relationships among these populations with certainty. Nonetheless, the overall phylogeny (Fig. 4) supports the contention that oviparity is the primitive condition and that oviparity has not reevolved from viviparity (Shine, 1985). Hence, our phylogeny indicates that viviparity has evolved within the species *L. bougainvillii.* This means that viviparity has evolved at least twice within the genus *Lerista,* once in the lineage leading to *L. microtus* and one or more times within the species *L. bougainvillii.* This conclusion is based on the following data:

(i) Viviparous and oviparous populations of *L. bougainvillii* are genetically similar (genetic distance of approximately 5%), whereas the viviparous *L. bougainvillii* are divergent from *L. microtus* (genetic distance of 25%). Indeed, the viviparous *L. bougainvillii* are more distant genetically from *L. microtus* (in terms of mitochondrial cyt *b*) than are most oviparous *L. bougainvillii.* Furthermore, placing a topological constraint on a search to support a hypothesis of a single origin of viviparity between these two species produced trees that required 22 steps more than the most parsimonious tree.

(ii) Populations of *L. bougainvillii* from Flinders Island, Chappell Island, and Tasmania branched off

together in all analyses, and they are genetically so similar that the relationship between them was unresolved. This close relationship indicates that the viviparous Bass Strait Island populations represent a single clade and thus are likely to have stemmed from a single origin of viviparity. The monophyletic nature of the Bass Strait populations supports the hypothesis that these island populations were formerly united by a continuous land bridge during a period of lowered sea levels (Rawlinson, 1974). However, rafting of individuals among islands is also possible and is equally consistent with the observed genetic homogeneity of the lizards on different islands.

(iii) Whether this Bass Strait group represents a separate origin of viviparity from the viviparous Kangaroo Island population is less clear. This question cannot be answered with any certainty with our data set, as there was not enough phylogenetic information in the sequence studied to resolve the relationships of the viviparous and oviparous populations from the center of the range.

The mtDNAs from the Woodside (Gippsland) population, which show an intermediate reproductive mode, cluster with other Victorian egg-laying populations. The fact that the eggs are only partially shelled, and contain late stage embryos, can be interpreted in two ways (Qualls *et al.*, 1995). If viviparity evolves through a gradual increase in the length of egg retention (e.g., Packard *et al.*, 1977; Shine and Bull, 1979), then the Gippsland lizards could be in the final stages of evolving viviparity. Alternatively, they could represent a hybrid population derived from viviparous and oviparous populations. Recent research on *Lacerta vivipara* is consistent with the latter explanation. When oviparous and viviparous *L. vivipara* are hybridized, the F_1 hybrids produce eggs that are only partially shelled and contain late-stage embryos (Heulin *et al.*, 1993), as in the Gippsland population of *L. bougainvillii.* Although sample sizes are small, the absence of mtDNAs characteristic of Bass Strait live-bearers within the Woodside population suggests that the intermediate condition is unlikely to be the result of recent (bidirectional) hybridization.

These groups have all diverged and remain genetically isolated; whether they have diverged enough to be called "species" depends upon the criteria chosen. However, if *L. bougainvillii* is to be broken up into separate species or subspecies it can only be done on the basis of phylogenetic divergence due to geographic isolation (e.g., Eyre Peninsula vs central populations and, perhaps, NSW). Based on the phylogeny found in our study, it would be illogical to separate *L. bougainvillii* into two or more species based on their mode of reproduction. Oviparous and viviparous populations of *L. bougainvillii* within the central part of the species range are more closely related to each other than either is to lizards from the periphery of the species range. Further, there are no morphological or electrophoretic characters to distinguish these lineages (Qualls *et al.*, 1995). Thus, *Lerista bougainvillii* satisfies any reasonable criteria for intraspecific bimodality in mode of reproduction—regardless of whether one's definition of "species" is based on the biological species concept (as evaluated by phenetic similarity; Qualls *et al.*, 1995) or the phylogenetic species concept (this study).

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