

Parrot Evolution and Paleogeographical Events: Mitochondrial DNA Evidence

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We sequenced 1,771 bp of three mitochondrial genes (12S rDNA, 16S rDNA, and cytochrome *b*) of nine New World parrot genera (*Amazona aestiva*, *Anodorhynchus hyacinthinus*, *Ara ararauna*, *Aratinga aurea*, *Cyanopsitta spixii*, *Derophtus accipitrinus*, *Guaruba guarouba*, *Pionus menstruus*, and *Pyrrhura picta*) and compared them with the corresponding sequence of Australian parakeet (*Melopsittacus undulatus*). Phylogenetic analyses (maximum parsimony and maximum likelihood) showed that the Neotropical species we studied constitute two monophyletic groups: the long-tailed and the short-tailed species. The separation within the long-tailed species could be assigned to the late Oligocene–early Miocene, when paleoenvironmental changes might have influenced this radiation. The long-tailed Neotropical and Australasian species diverged during the Cretaceous–Tertiary boundary, when South America and Australia were moving away from Antarctica (Gondwanaland fission). We also compared our data with the cytochrome *b* sequences of seven different genera of Australasian parrots obtained by other investigators, and these comparisons also support the independent evolution of the Neotropical and Australasian species. Analyses performed with 567 bp of partial sequences of 12S rDNA and cytochrome *b* did not support or refute the hypothesis of monophyly of the Neotropical parrots with respect to an African species whose sequences were available. However, this analysis supported the view that the divergence between Neotropical short- and long-tailed taxa was older than the Oligocene–Miocene divergence among the long-tailed genera.

Introduction

According to Forshaw (1989), there are more than 330 living species of parrots, mainly distributed in the Southern Hemisphere. One hundred species occur in South America, 69 of them in Brazil (Sick 1993, p. 264). Cracraft (1973) considers that the amount of evolution among parrots in Australia and South America “. . . suggests that the early history of the family was Gondwanaland, and therefore that paleogeography of the Southern continents was probably important.”

Parrot fossils are scarce; the oldest known is *Archeopsittacus verreauxi* (Upper Oligocene or Lower Miocene, about 30 MYA), found in France. The earliest modern genus representative is *Conuropsis fratercula* (Upper Miocene, around 20 MYA), found in the U.S.A. In South America, only Pleistocene (less than 2 MYA) fossils have been found, in Argentina, Ecuador, and Brazil (Forshaw 1989; Sick 1993, p. 251). The New and Old Worlds share no genera. Karyotype studies show the homogeneity among the South American species compared to the diversity among those found in Australasia (Lucca, Shirley, and Lanier 1991). DNA hybridization data (Sibley and Alquist 1990) suggest that there are three subgroups of parrots: Australasian, African, and American species. These results support the suggestion that New World parrots diverged from the other groups (Smith 1975; Sick 1993, p. 251).

Parrot phylogeny is better known among the Australasian species. Analysis of mitochondrial DNA (mtDNA) restriction fragment polymorphism carried out

on six species of the Australasian genus *Platycercus* revealed the presence of two groups and suggested the existence of a third (Overden, Mackinlay, and Crozier 1987). Leeton et al. (1994) sequenced 924 bp of the mitochondrial cytochrome *b* gene of eight Australasian parrots, including one considered extinct (*Geopsittacus occidentalis*), and established phylogenetic relationships among them. A shorter sequence of the same gene (307 bp) was compared among two African (*Poicephalus senegalus* and *Psittacus erithacus*), four Australasian (*Cacatua goffini*, *Melopsittacus undulatus*, *Nymphicus hollandicus*, and *Purpureicephalus spurius*), and six American species (*Amazona autumnalis autumnalis*, *A. oratrix oratrix*, *A. tucumana*, *A. xanthops*, *Graydidascalus brachyurus*, and *Pionus senilis*) (Birt et al. 1992). In this study, two groups were analyzed separately: the first included all the African and Australasian species, one American species (*Amazona ochrocephala*), and the common pigeon (which was considered as the out-group), while the second included all the American and one Australian species. Some consistent results were obtained: the African species were placed in one group, and three species of the American genus *Amazona* also formed a monophyletic group, while the fourth species (*Amazona xanthops*) seemed to diverge from the other species in the genus.

The information on the relationships among New World parrot species is scarce; the species can be separated according to their tail morphologies as long or short (Sick 1993, p. 703). These two groups can also be recognized by their peculiar behavior: long-tailed parrots generally live in the midstrata of trees and usually fly down to feed on the ground, while short-tailed parrots inhabit the canopy and are rarely seen on the ground (Montón 1977). The karyotypic characteristics of some long-tailed species also separate them from short-tailed ones (Valentine 1990). Another distinguishing feature is

Key words: parrots (Psittaciformes, Aves), mitochondrial DNA, evolution of parrots, paleogeography.

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Mol. Biol. Evol. 15(5):544–551, 1998

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Table 1
Primers Used

Gene ^a	Location ^b	Sequence	Reference
12S rDNA L	1735	GGATTAGATACCCCACTATGC	c
12S rDNA H	2170	AGGGTGACGGGCGGTATGTACG	c
16S rDNA L	2702	CCTACCGAGCTGGGTGATAGCTGGTT	c
16S rDNA H	3309	TGCGCTACCTTCGCACGGT	c
Cytochrome <i>b</i> L (universal)	14957	AAAGTAGTACGACTCTACACCTACCTTCGAAAAA	Kocher et al. (1989)
Cytochrome <i>b</i> H (universal)	15331	AAACTGCAGCCCCTCAGAATGATATTTGTCTCA	Kocher et al. (1989)
Cytochrome <i>b</i> H (long)	16092	AACTGCAGTCATCTCCGGTTTACAGAC	Kornegay et al. (1993)
Cytochrome <i>b</i> L (reverse)	15298	TGAGGCCAAATATCATTCTGAGGGGC	Cheng, Higuchi, and Stoneking (1994)
Cytochrome <i>b</i> L (internal)	15637	AACCTCCTAGGAGACCCAGAAAACCTTC	This work
Cytochrome <i>b</i> H (internal 2)	15764	CCTCTAGTTTGTGGGGATTGA	This work
Cytochrome <i>b</i> H (internal 3)	15417	GTCGGGTTGTCTACGGAGA	This work

^a L and H stand for the light and heavy chains, respectively.

^b Location in the chicken map (Desjardins and Morais 1990).

^c Designed by T.B. for another work.

the presence of minisatellite sequences detected by probe 33.15 (Jeffreys, Wilson, and Thein 1985) and linked to the W chromosome that are observed exclusively in long-tailed species (Miyaki et al. 1992, 1993, 1995, 1997).

In the present work, we sequenced regions of three mitochondrial genes (12S rDNA, 16S rDNA, and cytochrome *b*) from nine species of Neotropical parrots belonging to nine different genera. These data, along with previously published sequences (Birt et al. 1992; Leeton et al. 1994; Cooper and Penny 1997; Moore and DeFilippis 1997), were used to assess the time of divergence of these genera by applying substitution rate tests and maximum-likelihood methods.

Materials and Methods

The Neotropical species studied belong to aviculturists and official institutions in Brazil and can be morphologically distinguished as short-tailed (*Amazona aestiva* and *Pionus menstruus*) and long-tailed (*Anodorhynchus hyacinthinus*, *Ara ararauna*, *Aratinga aurea*, *Cyanopsitta spixii*, *Derophtus accipitrinus*, *Guaruba guarouba*, *Nandayus nenday*, and *Pyrrhura picta*). Blood samples were collected by venipuncture. Budgerigar (*Melopsittacus undulatus*) feathers were obtained from an aviculturist in England. Total DNA of two individuals of each species was extracted (Bruford et al. 1992).

Polymerase chain reaction (PCR) was used to amplify parts of three mitochondrial genes: 12S rDNA (436 bp), 16S rDNA (608 bp), and cytochrome *b* (1.1 kb). The amplifications were performed in 10- μ l volumes of 67 mM Tris (pH 8.8), 16.6 mM (NH₄)₂SO₄, 6.7 mM MgCl₂, 10 mM 2-mercaptoethanol, 10 mM EDTA (pH 8.8), 1.5 mM of each dNTP, 0.1 mg of BSA, 1–20 ng genomic DNA, 1 μ M of each primer, and 0.2–2.5 U of *Taq* polymerase (Advanced Biotechnologies Ltd.).

Primers used are listed in table 1. The products were isolated in 1% agarose in TBE buffer, PEG-recovered (Zhen and Swank 1993), and purified by phenol:chloroform extraction. This DNA was used as template, and the sequencing reaction was prepared according to the *Taq* dye deoxy terminator cycle sequencing kit man-

ufacturer (Applied Biosystems). Cytochrome *b* internal primers (table 1) were designed to cover both strands of the amplified region. After the sequencing reaction was carried out, the products were purified with phenol:chloroform:isoamyl alcohol (Applied Biosystems) and run in an automatic sequencer (ABI 373).

Since the partial *M. undulatus* cytochrome *b* sequence has already been published (Leeton et al. 1994), we sequenced only 116 bp of this gene and the 12S and 16S rDNA regions to adjust the alignment with the other New World species and *Gallus gallus* (outgroup; Desjardins and Morais 1990).

The DNA sequences obtained were visually aligned with multiple sequence editors (Sequence Navigator, Applied Biosystems and SeqPup; Gilbert 1996). The sequences of the genes (12S and 16S rDNAs and cytochrome *b*) were phylogenetically analyzed contiguously, as if they were a single gene. The phylogenetic contribution of each gene separately was also evaluated.

Cytochrome *b* sequences (885 bp) of the Neotropical parrots listed above were compared with those of Australasian parrots (*Geopsittacus occidentalis*, *Melopsittacus undulatus*, *Neophema petrophila petrophila*, *Pezoporus wallicus wallicus*, *Platycercus icterotis xanthogenys*, *Polytelis anthopeplus westralis*, and *Strigops habroptilis*; Leeton et al. 1994) and a woodpecker (*Colaptes rupicola*) (outgroup; Moore and DeFilippis 1997).

We also analyzed the partial sequences of 12S rDNA and cytochrome *b* (567 bp in total) of the Neotropical parrots, two Australasian parrots (*M. undulatus* and *S. habroptilis*; Leeton et al. 1994; Cooper and Penny 1997), one African parrot (*Psittacus erithacus*; Birt et al. 1992; Cooper and Penny 1997), and our outgroup (*G. gallus*; Desjardins and Morais 1990).

The final analysis comprised a comparison of 267 bp from cytochrome *b* of 2 African, 10 Australasian, and 15 Neotropical parrot species (Birt et al. 1992; Leeton et al. 1994; this work), chicken (Desjardins and Morais 1990), and *Columba livea* (Birt et al. 1992).

Phylogenetic analyses of the sequences were performed according to the following strategy: the preliminary search for the most-parsimonious tree was done using PAUP, version 3.1.1 (Swofford 1993). The same

program then calculated the strict consensus. A bootstrap test was performed (resampling of the original sequences' sites 100 times with reposition; for each sample, a most-parsimonious tree was estimated by heuristic search with 10 different starting orders to avoid spurious trees). The decay index of Bremer (1994) was calculated for each node of the consensus tree using the Autodecay program by Eriksson and Wikström (1995), together with PAUP.

The same data were used for a maximum-likelihood search using PAML (Yang 1997). This method uses an explicit model of nucleotide evolution that allows the comparison of parameter estimation among different sets of analyses. We applied the Tamura and Nei (1993) model, which was developed for mitochondrial DNA evolution that is characterized by its high A-T content and high transition/transversion rate. The heterogeneity of substitution rates (as that for synonymous vs. nonsynonymous substitution rates in the case of cytochrome *b* and that for conserved vs. variable sites, observed in almost any DNA sequence) was considered by distributing the rates in a discrete gamma distribution within eight categories. The alpha parameter, which is related to the shape of the gamma distribution, and the transition/transversion parameters were estimated from the data.

Rate constancy tests were done following the method described by Takezaki, Rzhetsky, and Nei (1995), using Takezaki's LINTREE programs. The taxa that presented significantly different rates (at the 5% level) were excluded from further analyses. The remaining taxa were reanalyzed by the maximum-likelihood method to obtain a tree that was constrained to behave as if it were directed by a molecular clock (in which all root-to-tip lengths have the same values). The analyses were performed in the group of species with the 1,771-bp sequences (12S and 16S rDNAs and cytochrome *b*) and the one with 885 bp of cytochrome *b* sequence.

Results

We obtained 342 bp of DNA sequence from the 12S rRNA genes and 428 bp from the 16S rRNA genes (GenBank accession numbers are presented after species names) from 10 species of Neotropical parrots (*A. aestiva*—U70738 and U70749, *A. hyacinthinus*—U70741 and U70752, *A. ararauna*—U70739 and U70750, *A. aurea*—U70740 and U70751, *C. spixii*—U70742 and U70753, *D. accipitrinus*—U70743 and U70754, *G. guarouba*—U70744 and U70755, *N. nenday*—U70746 and U70757, *P. menstruus*—U70747 and U70758, and *P. picta*—U70748 and U70759, respectively) and from the Australian parakeet (*M. undulatus*—U70745 and U70756). We also sequenced 1,005 bp of the cytochrome *b* genes of nine species of New World parrots (*A. aestiva*—U70760, *A. hyacinthinus*—U70763, *A. ararauna*—U70761, *A. aurea*—U70762, *C. spixii*—U70764, *D. accipitrinus*—U70765, *G. guarouba*—U70766, *P. menstruus*—U70767, and *P. picta*—U70768). Two individuals per species were studied.

Table 2
Frequencies of Transitions and Transversions Among Variable Sites

Gene ^a	Transitions	Transversions	Total	Ratio ^b
12S rDNA	1,270 (80%)	316 (20%)	1,584	4:1
16S rDNA	2,415 (46%)	2,840 (54%)	5,255	1:1
Cytochrome <i>b</i> (N) . .	5,554 (66%)	2,892 (34%)	8,446	2:1
Cytochrome <i>b</i> (N-A)	11,581 (57%)	8,826 (43%)	20,407	1.3:1

^a N and A stand for the New World and Australasian genera, respectively.

^b Transitions : transversions.

Since the first tree constructions always showed individuals of the same species grouped together, we considered just one sequence per species in the phylogenetic analyses to speed up the computer analyses.

The alignment showed that insertions and/or deletions ("indels") were present only in 12S and 16S rDNA genes (ranging from 20 indels in *D. accipitrinus* to 34 in *P. picta*); the cytochrome *b* fully aligned. The composition of the sequences showed a higher frequency of cytosine and adenine. The number of transitions observed was higher than the number of transversions at the variable sites studied (table 2). As expected, the cytochrome *b* third position was the most variable position.

The pairwise distances among the 1,771-bp sequences were calculated according to the model of Tamura and Nei (1993) and are presented in table 3. The average kappa parameters (reflecting the overall transition/transversion substitution rates) are also presented in table 3. The phylogenetic trees resulting from the analyses of each gene separately for the Neotropical parrots plus *M. undulatus* and *G. gallus* showed that the relationships (associations and/or divergences) obtained using the 12S rDNA sequences were not always totally in accordance with the relationships obtained using the 16S rDNA and cytochrome *b* sequences, which resulted in similar trees (data not shown).

Two shortest trees of equal length (1,561 steps) were obtained in parsimony analyses of the 1,771 bp involving the nine Neotropical species, *M. undulatus*, and *G. gallus*. The strict-consensus and maximum-likelihood trees are presented in figure 1. These trees show that the polytomy obtained in the parsimony analysis corresponds to very short branch lengths in the maximum-likelihood analysis. We also performed neighbor-joining analyses using a distance matrix, and these showed almost the same tree patterns as those obtained with the maximum-likelihood analyses (data not shown).

The parsimony and maximum-likelihood trees of the 885-bp cytochrome *b* sequence from the Neotropical and Australasian species are shown in figure 2. The analyses using the combined sequences of 12S rDNA and cytochrome *b* resulted in the parsimony and maximum-likelihood trees presented in figure 3. The phylogeny obtained by a maximum-parsimony heuristic search of 267 bp of the cytochrome *b* gene is shown in figure 4.

We tested the constancy of evolutionary rate of the three mitochondrial gene sequences of each New World

Table 3
Pairwise Distances Among the 1,771-bp Sequences

	<i>Gallus gallus</i>	<i>Melop-sitt-acus undulatus</i>	<i>Amazona aestiva</i>	<i>Anodor-hynchus hyacinthinus</i>	<i>Ara ararauna</i>	<i>Aratinga aurea</i>	<i>Cyanopsit-ta spixii</i>	<i>Deropterys accipitrinus</i>	<i>Guaruba guarouba</i>	<i>Pionus menstruus</i>	<i>Pyrrhura picta</i>
<i>G. gallus</i>		0.332	0.292	0.321	0.324	0.300	0.362	0.302	0.316	0.280	0.305
<i>M. undulatus</i>	2.02		0.262	0.261	0.296	0.283	0.317	0.268	0.288	0.243	0.266
<i>A. aestiva</i>	2.17	2.85		0.184	0.179	0.179	0.251	0.174	0.186	0.112	0.182
<i>A. hyacinthinus</i>	2.03	2.59	5.87		0.122	0.112	0.199	0.131	0.121	0.173	0.140
<i>A. ararauna</i>	2.18	3.43	5.65	10.60		0.114	0.201	0.134	0.121	0.175	0.145
<i>A. aurea</i>	2.06	3.20	6.80	11.10	11.78		0.181	0.126	0.102	0.164	0.131
<i>C. spixii</i>	1.96	3.08	3.56	4.49	4.54	4.56		0.214	0.203	0.250	0.232
<i>D. accipitrinus</i>	2.32	3.19	6.41	9.08	9.65	10.99	4.53		0.128	0.158	0.130
<i>G. guarouba</i>	2.30	3.35	6.11	10.29	9.75	12.13	4.54	11.01		0.157	0.138
<i>P. menstruus</i>	1.97	2.94	10.72	5.61	5.69	5.73	3.75	5.69	5.33		0.156
<i>P. picta</i>	2.19	2.68	5.82	9.48	11.34	10.69	4.86	8.10	11.23	4.84	

NOTE.—The distances were calculated according to the Tamura and Nei (1993) correction, with alpha set to 1.0. The distances are presented above the diagonal. The kappa parameters are presented below the diagonal.

species plus *M. undulatus* and *G. gallus* using the two-cluster and branch length tests in LINTREE with the Tamura and Nei (1993) distance. *Cyanopsitta spixii* had a faster rate and *A. aestiva* and *P. menstruus* had slower ones than did the other species. None of these species were included in the time-constrained tree. Assuming that bird orders diverged around 100 MYA (Hedges et al. 1996; Cooper and Penny 1997) and using the time-constrained tree (fig. 5), we estimate the date of the split between the Neotropical long-tailed parrot genera and *M. undulatus* to be around 76 MYA (table 4) and the separation of these New World genera to be between 27 and 20 MYA. The standard errors in table 4 were calculated using the time-constrained maximum-likelihood data.

The 885-bp cytochrome *b* time-constrained tree was also obtained through the PAML program. The evolutionary rates of *A. ararauna*, *D. accipitrinus*, *G. guarouba*, *P. menstruus*, *P. icterotis*, *P. anthoepus*, and *S. habroptilis* differed too much from those of the remaining 10 genera for them to be included in the time-constrained tree (fig. 6). If the separation of the Neotropical

and Australasian species occurred around 76 MYA (table 4), the split among the New World long-tailed parrots can be dated at between 51 and 24 MYA (table 4).

Discussion

The high frequency of cytosine and adenine in the composition of the three genes studied is similar to that found in the total mitochondrial sequence of chicken (*G. gallus*; Desjardins and Morais 1990). The cytochrome *b* sequences obtained showed a higher frequency of cytosine at the first position. At the second position, there was more thymidine and less guanine, while in the third position, adenine and cytosine were more highly represented. In the Neotropical parrots, transitions are responsible for most of the nucleotide variation in the three genes studied. Other authors observed the same trend in other organisms (Brown et al. 1982; Desjardins and Morais 1990). The highest variability at the third position of a mitochondrial coding gene has been observed in a variety of other organisms (Edwards, Arcander, and Wilson 1991; Ramirez, Savoie, and Morais 1993; Krajewski and Fetzner 1994) and can be explained by the degenerated coding system (silent substitutions). For this reason, some authors have chosen

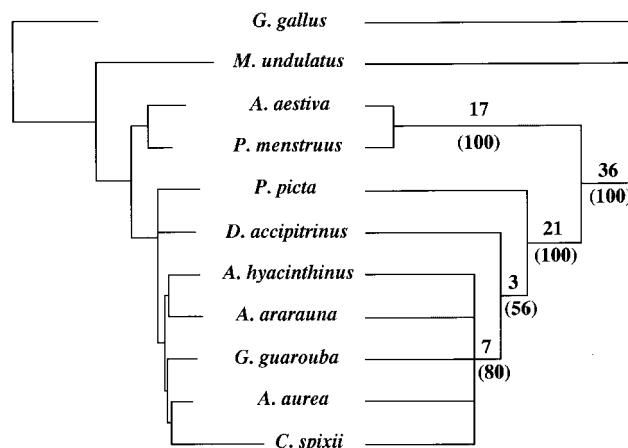


FIG. 1.—Trees obtained using 1,771 bp of cytochrome *b*, 12S rDNA, and 16S rDNA sequences. Maximum-likelihood tree on the left. Strict-consensus maximum-parsimony tree on the right, showing Bremer decay indices at each branch and bootstrap values in parentheses.

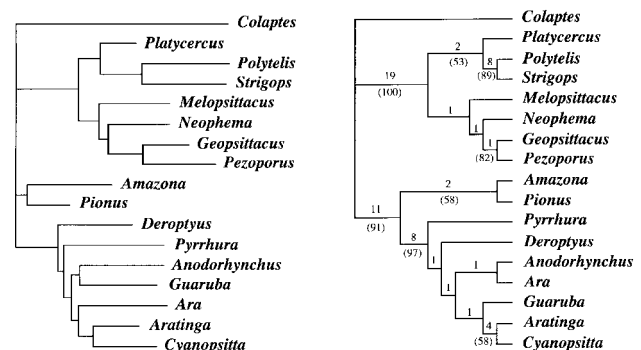


FIG. 2.—Trees obtained using 885 bp of cytochrome *b* sequence. Maximum-likelihood tree on the left. Maximum-parsimony tree on the right, showing Bremer decay indices at each branch and bootstrap values in parentheses.

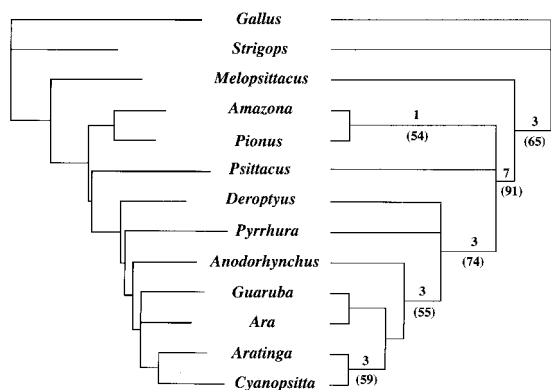


FIG. 3.—Trees obtained using 567 bp of 12S rDNA and cytochrome *b* sequences. Maximum-likelihood tree on the left. Maximum-parsimony tree on the right, showing Bremer decay indices at each branch and bootstrap values in parentheses.

not to include this position in their phylogenetic analyses (Avice, Nelson, and Sibley 1994). Our results showed that there are some differences among the trees if the third position is not considered, but the most consistent relationships (associations and/or divergences) are not affected (data not shown).

The trees based on the 12S rDNA sequence did not agree very well with the trees based on the other two sequences. This could be due to the small size of the sequence, since the analyses based on a longer sequence of the 16S rDNA gene resulted in trees similar to the ones obtained with cytochrome *b*.

The trees resulting from analyses of the 1,771-, 885-, and 638-bp mitochondrial sequences presented some high values of bootstrap and Bremer decay. However, the 267-bp cytochrome *b* comparisons produced very low values, indicating that relationships obtained

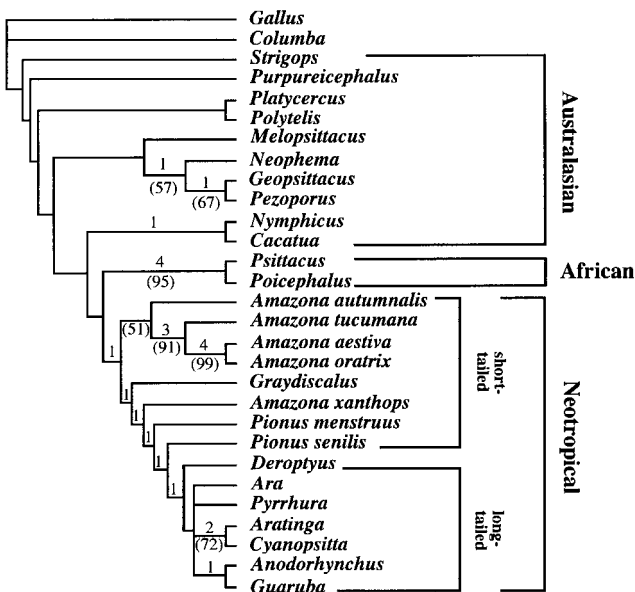


FIG. 4.—Maximum-parsimony tree obtained using 267 bp of cytochrome *b* sequence. Bremer decay indices are shown over branches, and bootstrap values that were higher than 50 out of 100 replicates are shown in parentheses.

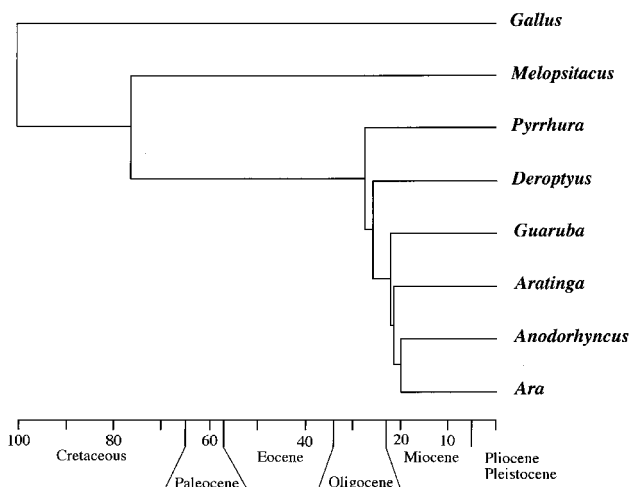


FIG. 5.—Time-constrained tree obtained using 1,771 bp of cytochrome *b*, 12S rDNA, and 16S rDNA sequences.

using this sequence are not reliable. Thus, the data set with the smaller sequences was not analyzed by maximum likelihood.

The monophyly of Neotropical parrots with respect to the Australasian ones is supported by the analyses using all four sequences (the 1,771-bp sequences of the three mitochondrial genes, the 885-bp cytochrome *b* sequences, the 567-bp 12S rDNA and cytochrome *b* comparisons, and the 267-bp of cytochrome *b* sequences). Allozyme (Randi and Bertagnolio 1990) and DNA hybridization (Sibley and Alquist 1990) studies also support separation of the parrots from Australasia, Africa, and South America. Cracraft (1974) suggested that parrots and other groups of birds may have had their dispersal influenced by the Gondwanaland connections since: (1) there is evidence that a flightless ratite ancestor population was present in Gondwanaland, possibly

Table 4
Estimated Dates of Separation of Parrot Species

Species	Date ± SE (MYA)
1,771 bp of 12S rDNA, 16S rDNA, and cytochrome <i>b</i> sequences	
M × P, D, G, Au, Ah, Ar	76.2 ± 7.8
P × D, G, Au, Ah, Ar	27.4 ± 2.2
D × G, Au, Ah, Ar	25.7 ± 2.0
G × Au, Ah, Ar	22.0 ± 1.7
Au × Ah × Ar	21.2 ± 1.7
Ah × Ar	19.7 ± 1.7
885 bp of cytochrome <i>b</i> sequences	
M × N, Go, Pw	36.2 ± 3.5
N × Go, Pw	34.5 ± 3.4
Go × Pw	23.8 ± 2.9
A × P, Ah, Au, C	50.8 ± 4.6
P × Ah, Au, C	37.2 ± 3.4
Ah × Au, C	32.5 ± 3.2
C × Au	24.0 ± 2.9

NOTE.—M, *Melopsittacus undulatus*; P, *Pyrrhura picta*; D, *Deroptylus accipitrinus*; G, *Guaruba guarouba*; Au, *Aratinga aurea*; Ah, *Anodorhynchus hyacinthinus*; Ar, *Ara ararauna*; A, *Amazona aestiva*; C, *Cyanopsitta spixii*; N, *Neophema petrophila*; Pw, *Pezoporos wallicus*; Go, *Geopsittacus occidentalis*, respectively.

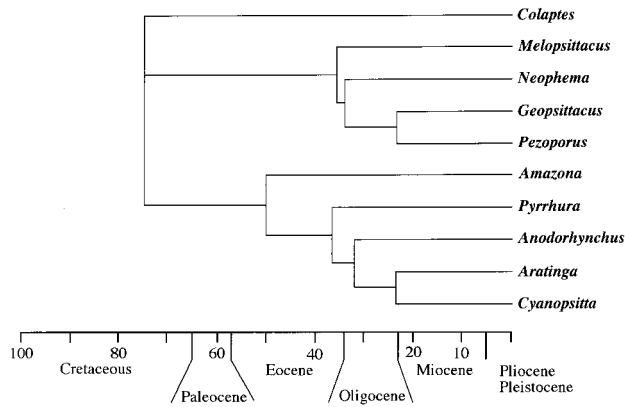


FIG. 6.—Time-constrained tree obtained using 885 bp of cytochrome *b* sequences.

isolated into a cassowary/emu group in Australia, rhea in South America, and ostriches in Africa and Eurasia (before the Quaternary); (2) it is possible that galliforms occurred in Australia, East Antarctica, and South America, from where they colonized Laurasia; and (3) the presence of five genera of penguins on Seymour Island (Antarctic Peninsula) related (in various degrees) to New Zealand genera could be explained by the presence of a common ancestor when New Zealand and West Antarctica were close or in contact. Gondwanaland was an antique continent that split during the late Mesozoic and early Tertiary into South America, Africa, Australia, New Zealand, Antarctica, and India. Glenny (1954) even suggested that the origin and center of dispersion of parrots might be assigned to the Antarctic region.

The date of the separation of *M. undulatus* (Australian parakeet) and the long-tailed Neotropical species we estimated using the 1,771-bp comparisons (76 MYA) is in accord with the hypothesis that continental drift played an important role in the evolution of many animals and plants. These paleogeographic continental movements could have been responsible for the isolation of groups of parrots that then evolved independently.

The datings we obtained were based on estimates of the divergence of bird orders suggested by Hedges et al. (1996) and Cooper and Penny (1997). We considered 100 MYA as a conservative estimate for the separation of Galliformes and Psittaciformes (if more accurate dating becomes available in the future, our analyses can be linearly corrected). We observed that the 885-bp comparisons gave older dates than those obtained using the 1,771-bp sequences (table 4). A comparison of kappa parameters for the different data sets (table 5) showed that the analysis involving only the cytochrome *b* se-

quences underestimated the transition/transversion rates. As can be seen in table 3, the smaller distances between sequences correspond to higher values of the transition/transversion rate parameters, because they are estimates closer to the instantaneous rates. The rate parameters obtained in the cytochrome *b* analyses are lower than those obtained in other analyses, which leads to an over-estimation of the separation dates. Thus, the datings obtained with the larger data set seem more reliable.

The separation of the long- and short-tailed Neotropical parrots observed in the trees shown in figures 1–3 is also supported by other data: morphology (Sick 1993, p. 264), minisatellites (Miyaki et al. 1992, 1993, 1995, 1997), karyotype (Valentine 1990), and behavior (Montón 1977). The mitochondrial sequences of these short-tailed species evolved more slowly than did those of the long-tailed ones. This suggests that these two groups of Neotropical parrots evolved independently. The divergence between the long- and short-tailed parrots could not be geographically assigned to South America or Africa, since in the 567-bp analyses that included parrots from the three continents (fig. 3), the Neotropical genera were not monophyletic with respect to the African taxa.

Among the long-tailed New World species, *P. picta* and *D. accipitrinus* probably diverged earlier from the others in the group. These species are exceptions among the long-tailed Neotropical parrots regarding the existence of W-chromosome-linked minisatellite sequences (Miyaki et al. 1997). This result adds more data supporting the lack of close relationships between these two species and the rest of the long-tailed parrot taxa. In this context, the W-chromosome-linked minisatellite sequence was acquired after the separation of the monophyletic branch of the long-tailed parrots from *P. picta* and *D. accipitrinus*.

The relationships among the long-tailed New World parrots could not be well established. The association of *C. spixii* and *A. aurea* was the only one observed in all trees (figs. 1–4) except one of the two shortest maximum-parsimony trees. This suggests that the Spixii's macaw is more related to a conure than to a macaw. Based on morphological studies, Sick (1993, p. 261) also suggested that *C. spixii* is not a true macaw.

The lack of resolution in relationships among the long-tailed Neotropical genera could be an inherent consequence of the nature of our data, whereby the phylogenetic signal has been lost by saturation. In this case, a slow-evolving gene would be more suitable for comparisons. Alternatively, the diversification of these taxa might have occurred almost simultaneously. The esti-

Table 5
Estimated Parameters from the Maximum-Likelihood Analyses

Analysis	K1	K2	Alpha
1,771 bp (12S and 16S rDNAs, cytochrome <i>b</i>)	9.02244 (0.88942)	5.17000 (0.55864)	0.33938 (0.02408)
885 bp (cytochrome <i>b</i>)	4.89729 (0.40380)	4.19789 (0.43546)	0.42362 (0.03315)
567 bp (12S rDNA, cytochrome <i>b</i>)	12.67598 (2.01750)	4.25987 (0.69954)	0.23321 (0.02723)

NOTE.—K1 and K2 are parameters from the Tamura and Nei (1993) model related to transition rates. K1 estimates the purine transitions, and K2 estimates the pyrimidine transitions. The standard errors of the estimates are in parentheses.

mated date for this diversification event was between 27 and 20 MYA based on 1,771 bp of 12S and 16S rDNAs and cytochrome *b*. It is clear that these radiation events occurred during the late Oligocene–early Miocene. Unfortunately, studies on the South American paleoenvironment during this period are not very detailed.

It is known that during the Oligocene (ca. 30 MYA), there was a regression of the sea level, and at the transition of this period to the Miocene, there was a huge transgression (Petri and Fúlvaro 1983). Orogenic movements were most active around 30 MYA and characterized the division of the Paleogene and Neogene (Salgado-Labouriau 1994). The Oligocene–Miocene boundary is associated with the beginning of a South American transcendental biotic change which led to the vanishing or disappearance of most “primitive” Paleogene mammals (Pascual and Jaureguizar 1990). Fossil records of immigrant primates and rodents from Africa are also dated to this time (Poirier, Stini, and Wreden 1994). Also based on fossil data, it was suggested that the autochthonous platyrrhini monkeys radiated in the pre-middle Miocene, which may be related to an early–middle-Miocene climatic amelioration and expansion of the forest/riparian habitat (Flynn et al. 1995). The congruence of the dating for the long-tailed parrots’ divergence and the radiations of primates and other mammals suggest that changes in the paleoenvironment may have influenced the diversification of these groups of animals.

Most of the Neotropical parrot genera that we studied are distributed over large ranges. Only *Guaruba* and *Cyanopsitta* can be considered endemic in a strict sense and their differentiation probably occurred independent of (or prior to) the physiographic or climatic barriers as we know them today. Thus, if the nearly simultaneous differentiation of various long-tailed genera detected here is not a consequence of technical limitations, it should provide evidence supporting abrupt climatic changes of continental range which could have expanded and contracted forests.

The final conclusions of the present work are that the divergence of Neotropical and Australasian species in the Cretaceous–Tertiary boundary and the separation among the long-tailed parrots during the late Oligocene–early Miocene can be explained by paleogeographic events.

Acknowledgments

We thank C. F. M. Menck, O. Hanotte, and I. Biasia for their constant support; N. Takezaki and M. Nei for their invaluable help; P. F. Flecha (Sociedade Ornitológica Bandeirante), A. Stocker, and F. M. C. F. Matioli for comments; A. R. Rogers and two anonymous reviewers for invaluable suggestions; E. A. Reis for helping us with computer facilities; and LCCA-USP for the use of their supercomputer. A. Marra, A. Vertematti, A. Kruppa, C. Isoldi, N. Kawall, L. Maluf, A. L. V. Nunes (Sorocaba Zoo), A. Estevan Filho (Americana Zoo), L. A. Labruna (Parque Ecológico do Tietê), A. M. B. Espuny (Divisão Técnica de Medicina Veterinária e Biologia da Fauna), N. M. R. Guedes (Projecto Arara-azul), G. V. Canali (Criadouro de Animais

Silvestres de Itaipu Binacional), and M. I. Bampi (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) kindly supplied the parrot samples. C.Y.M. has a BUNKA/90 prize. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Conselho Nacional de Pesquisa.

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ALAN R. ROGERS, reviewing editor

Accepted January 26, 1998