Molecular Phylogenetics and Evolution
Vol. 17, No. 1, October, pp. 108–116, 2000
doi:10.1006/mpev.2000.0821, available online at http://www.idealibrary.com on IDEAL®

A Molecular Framework for the Phylogeny of the Ant Subfamily Dolichoderinae

Maria Chiotis,* Lars S. Jermiin,† and Ross H. Crozier*,[1]

*Department of Genetics, La Trobe University, Bundoora, Victoria 3083, Australia; and †Australian Genomic Information Centre, Building C80, University of Sydney, New South Wales 2006, Australia

Received November 9, 1999; revised June 5, 2000

Partial sequences are reported for the mitochondrial genes for cytochrome oxidase subunits 2 and 3 and for cytochrome *b*, and the entire sequence of the gene for $tRNA^{Leu}_{UUR}$ for species from 14 genera of dolichoderine ants and from three outgroup genera. Considerable variation was observed between tRNA genes in the size of the TΨC arm and the DHU and anticodon loops and whether or not the TΨC stem possesses a GC pair. The outgroup taxa showed complete TAA CO1 stop codons, but dolichoderines have either TA or T. The outgroup taxa showed a noncoding gap between the *CO1* and the $tRNA^{Leu}_{UUR}$ genes. A phylogeny-independent compatibility test using the amino acid sequences showed differences between the genes consistent with variation in evolutionary rates, according with other studies. Base compositions proved heterogeneous between species, hence phylogenetic analysis was restricted to the protein sequences using maximum likelihood and the mtREV24 replacement matrix. A maximum-likelihood consensus tree has similarities to those from morphological studies with some exceptions such *Leptomyrmex* falling within the dolichoderine genera rather than basally, and the accretion of genera formerly included under *Iridomyrmex*. Features of the tRNA genes and the CO1 termination codons agree quite well with the molecular phylogeny.

Key Words: **ant tribe Dolichoderinae; mitochondrial DNA; cytochrome oxidase 1; cytochrome oxidase 2; cytochrome *b*; molecular phylogeny, maximum-likelihood consensus; $tRNA^{Leu}_{UUR}$ evolution; stop codon evolution.**

INTRODUCTION

Ants, with some 15,000 estimated species, form a large group of animals that are dominant in most terrestrial ecosystems (Wilson, 1992) and important in the study of evolutionary biology because of the high expression of their social behavior (Bourke and Franks, 1995; Crozier and Pamilo, 1996; Hölldobler and Wilson, 1990). The Dolichoderinae are, with 854 species (Shattuck, 1992a), the fourth largest ant subfamily (Bolton, 1995). Although they are much less diverse in life pattern than the third largest subfamily, the Ponerinae (Hölldobler and Wilson, 1990), they dominate the ant fauna in various parts of the world (e.g., Australia), and are significant components elsewhere. Noteworthy are species of *Azteca,* prominent members of the Neotropical mutualistic inhabitants of *Cecropia* plants, *Linepithema,* including the worldwide tramp pest *L. humile,* and *Technomyrmex,* including *T. albipes* with a complex alternation of dispersal types (Tsuji *et al.,* 1991). Fossil evidence indicates that the subfamily was even more dominant in the Oligocene (Brown, 1973).

The history of our understanding of the phylogeny and systematics of the Dolichoderinae is given by Shattuck (1992a, 1995). Briefly, the subfamily has been traditionally regarded as comprising one large tribe plus several small ones. Most recently in this tradition, Hölldobler and Wilson (1990) recognized one large tribe (the Tapinomini with 20 extant genera) and two small ones (the Dolichoderini with four extant genera and the Leptomyrmecini with one). Subsequently, Shattuck (1992a,c) found that tribal divisions within the subfamily are currently unsupportable and created several new genera, yielding the present muster of 22 extant genera.

Using 104 morphological characters and as outgroups genera of the related subfamilies Aneuretinae and Formicinae, Shattuck (1995) carried out two phylogenetic analyses, one with characters ordered (Fig. 3A) and one with characters unordered (Fig. 3B); all dolichoderine genera were included in these analyses except for the genus *Ecphorella* (known from a single specimen and hence unknowable for the full suite of characters). These results form an excellent framework for the study of dolichoderine behavior and systematics, but require testing and extension, especially given

[1] To whom correspondence should be addressed. Present address: School of Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia. E-mail: Ross.Crozier@jcu.edu.au.

1055-7903/00 $35.00

TABLE 1

Specimens Used and Their Origins

Species	Locality	Code/collection	Identification
Anonychomyrma sp.	Cape Shanck, Vic.	ACBN/RHC	SOS
Azteca cf *longiceps*	Santa Cruz, Bolivia	12296/PSW	PSW
Bothriomyrmex meridionalis	Montricoux, Tarn-et-Garonne, France	ACFC/BEK&AL	LP
Camponotus consobrinus	Camberwell, Vic.	ACBX/RHC	SOS
Dolichoderus nr lutosus	Santa Cruz, Bolivia	12304/PSW	PSW
Dorymyrmex insanus	Yolo Co., California	13028/PSW	PSW
Forelius chalybaeus	Tucuman, Argentina	12857-2/PSW	PSW
Iridomyrmex lividus	Fowler's Gap, NSW	ACBL.1/RHC	SOS
Leptomyrmex unicolor	Daintree, Qld	ACBW/BPO	SOS
Linepithema humile	Bundoora, Vic.	ACBS/MC	SOS
Liometopum occidentale	Solano Co., California	ACBP/RHC	PSW/SOS
Ochetellus sp.	Camberwell, Vic.	ACBM/RHC	SOS
Papyrius sp.	Bundoora, Vic.	ACLA/RHC	RHC
Paratrechina sp	Reservoir, Vic.	ACDN/BEK	SOS
Rhytidoponera sp 12	Fowler's Gap, NSW		RWT
Tapinoma sp.	Dandenongs, Vic.	ACBJ/RML	SOS
Technomyrmex sp.	Bundoora, Vic.	ACKZ/RHC	RHC

Note. Australian states: Vic, Victoria; NSW, New South Wales; Qld, Queensland. RHC, R. H. Crozier; BEK, B. E. Kaufmann; AL, A. Lenoir; LP, L. Passera; BPO, B. P. Oldroyd; SOS, S. O. Shattuck; RWT, R. W. Taylor; PSW, P. S. Ward. Letter codes refer to RHC's collection and number codes to PSW's collection. All species fall into the Dolichoderinae, except for the outgroup species *Camponotus consobrinus* and *Paratrechina* sp [Formicinae] and *Rhytidoponera* sp. 12 [Ponerinae].

that several nodes had very low confidence. Molecular phylogenetic studies of ants are relatively rare so far (Ayala *et al.,* 1996; Baur *et al.,* 1993, 1995, 1996; Crozier *et al.,* 1995; Wetterer *et al.,* 1998), although ant sequences have been included in studies of families and orders and, in combination with fossil dating, have yielded an estimate of the time of origin of the group as falling into the Jurassic (Crozier *et al.,* 1997). The only molecular phylogenetic study of dolichoderine ants was restricted to species of *Azteca* (Ayala *et al.,* 1996).

Here we use sequences from three mitochondrial genes to build on the work of Shattuck (1995) to establish a framework for the study of dolichoderine behavior and systematics. In particular, in addition to providing new information on the relationships between ant genera, we test the major morphological findings of a sister group relationship of *Leptomyrmex* to other dolichoderines and, in the context of the genera used, the existence of three groups of genera.

METHODS

Specimens

Genera were selected in order to sample the major groups recognized by Shattuck (1995) as shown in Figs. 3A and 3B, and to test some of the findings from morphology, such as the reorganization of the species formerly placed in *Iridomyrmex* (Shattuck, 1992c). Outgroup genera were selected from the subfamilies Formicinae (usually regarded as that closest to the Dolichoderinae (Shattuck, 1992b)) and the Ponerinae, a group often regarded as rather distant from the Dolichoderinae (Baroni Urbani *et al.,* 1992). For the purposes of this study, genera were accepted as monophyletic, although some in future may prove to be otherwise (Shattuck, 1995). The names of the species used and their provenances are given in Table 1.

Sequences

All sequences reported here are new except for the cytochrome *b* gene sequences for *Leptomyrmex unicolor* (Crozier *et al.,* 1997) and *Rhytidoponera* sp 12 (Tay *et al.,* 1997). Partial sequences were obtained from the mitochondrial *cytochrome b* and *cytochrome oxidase 1* and *2* genes, and the complete $tRNA^{Leu}_{UUR}$ sequence was obtained. Briefly, DNA was extracted with standard methods (CTAB and Chelex). Amplification for all sequences involved an initial 94°C step for 3 min, then 35 cycles of 92°C for 30 s, a 30-s annealing step, and 72°C for 30 s, using a Perkin-Elmer thermocycler. The annealing step varied according to species: 42–47°C for the cytochrome *b* region, 48–50°C for all uses of primer L3034, and 42–50°C for all uses of primer J2791. In addition, for sequences from *Anonychomyrma, Camponotus, Forelius, Liometopum,* and *Tapinoma* it proved necessary to amplify a larger region using primer Jerry, followed by using J2791 as an internal sequencing primer. Otherwise the combinations of primers used are given in Table 2; primers used in PCR were also used for direct dideoxy cycle sequencing following purification of PCR products using Promega's PCR Wizard Prep. Promega's fmol kit was used for sequencing.

Alignment proceeded by treating the genes sepa-

TABLE 2

PCR Primers Used

Region/name	Sequence	Position
Cytochrome oxidase 1 and 2		
J2791	ATACCTCGACGTTATTCAGA	3093–3112→
L3034	TAATATGGCAGATTAGTGCA	3357–3377→
H3389	TCATAACTTCAGTATCATTG	←3924–3943
H3665	CCACAAATTTCTGAACATTG	←4194–4213
C1-J-2183 (Jerry)	CAACATTTATTTTGATTTTTTGG	2481–2503→
C2-N-3389 (Marilyn)	TCATAAGTTCA(GA)TATCATTG	←3924–3943
C2-N-3661 (Barbara)	CCACAAATTTCTGAACATTGACCA	←4190–4213
Cytochrome *b*		
CB1	TATGTACTACCATGAGGACAAATATC	11400–11425→
CB2	ATTACACCTCCTAATTTATTAGGAAT	←11859–11884
CB3ext	CCTA(CT)TCATATTCAACCAGA(AG)TGA	11802–11825→
tRs2	GAAAATTTTATTTCTATATTAT(AG)TTTTCA	←12230–12258

Note. Position denotes coordinates in honeybee genome (Crozier and Crozier, 1993). Primer combinations: CB1–CB2, CB1–tRs2, CB3ext–tRs2, Jerry-H3665, Jerry-H3389, J2791–H3389, J2791–H3665, L3034–H3389, L3034–H3665. Primers J2791, L3034, H3389, and H3665 from A. T. Beckenbach (personal communication). Depending on species, Marilyn was substituted for H3389 and Barbara for H3665.

rately, using ClustalX (Thompson *et al.,* 1997) and Se-Al (http://evolve.zoo.ox.ac.uk/Se-Al/Se-Al.html). Determination of tRNA sequence was assisted by using tRNAscanSE (Lowe and Eddy, 1997).

Phylogenetic Hypotheses under Test

The hypotheses indicated on morphological grounds are derivable from Figs. 1 and 2 and can be stated in the context of the genera we have studied as (1) a basal position for *Leptomyrmex,* (2) a monophyletic group of *Dolichoderus, Liometopum, Tapinoma,* and *Technomyrmex,* (3) a monophyletic group of *Iridomyrmex, Ochetellus,* and *Papyrius,* and (4) a monophyletic group of *Bothriomyrmex, Dorymyrmex,* and *Forelius.*

Phylogenetic Treatment of Data

We carried out our phylogenetic analyses in a maximum-likelihood framework using the programs MolPhy 2.3b3 (Adachi and Hasegawa, 1996b) and PUZZLE 4.0.1 (Strimmer *et al.,* 1997). For comparative purposes we also performed an unweighted parsimony analysis using PAUP 3.1.1 (Swofford, 1993).

Heterogeneity Analysis

Nucleotide sequences for each codon position were obtained using MOLCODON from the MolPhy package. We tested for heterogeneity between base or protein sequences using NUCST and PROTST from the MolPhy package to obtain the numbers of each nucleotide or amino acid, and Monte Carlo RxC (W. R. Engels; Lewontin and Felsenstein, 1965) and PUZZLE 4.0.1 (Strimmer and von Haeseler, 1996).

Nonrandomness of the phylogenetic signal shown by different sections of the amino acid sequences was tested for using RETICULATE (Jakobsen and Easteal, 1996), which displays the compatibility with respect to phylogenetic signal for each site relative to all others.

Phylogenetic Analysis of the Data

We used the inferred amino acid sequences because (i) compositional homogeneity was rejected for the nucleotide sequences but not for the amino acid sequences, (ii) if stationarity is violated homoplasy can generate more similarity between distantly than between closely related taxa, so that they may sort out by base composition rather than by relationship (Lockhart *et al.,* 1994), and because (iii) for such deep divergences (Crozier *et al.,* 1997) phylogenetic analyses based on DNA sequences might be affected by correlations between codon positions (Adachi and Hasegawa, 1996b; Crozier and Crozier, 1993).

We concatenated the aligned amino acid sequences and tested the appropriate substitution model out of the Proportional (Felsenstein, 1981), Dayhoff (Dayhoff *et al.,* 1978), JTT (Jones *et al.,* 1992), and mtREV24 (Adachi and Hasegawa, 1996a) models. Because differences in the number of degrees of freedom cannot be determined for comparisons between these models, we used ProtML from the MolPhy package under each of the substitution models to analyze the data using the stepwise addition mode (-q option) and the resulting 120–140 trees were then used as seeds for local rearrangement searches (-R option). We then rearranged the order of sequences in the input file and repeated this procedure. The tree with the highest likelihood found so far was then identified and applied to each of 100 pseudo-replicates prepared using SeqBoot using each of the substitution models.

Having identified the most appropriate substitution model for the data we then made an additional eight randomizations of the input order, and used the -q option to obtain trees then used as seeds for the -R option. We took the resulting trees and restricted our attention to the most likely tree and the 22 other trees

which did not differ significantly at the 5% level from the tree with the highest likelihood according to the Kishino-Hasegawa test (Kishino and Hasegawa, 1989). From this set we derived a standardized exponentially weighted consensus tree following Jermiin *et al.* (1997). This tree was produced via the program TREECONS (Jermiin *et al.,* 1997), which produces output appropriate for the program CONSENSE from the Phylip package. Support for a group in this tree is not given by the number of trees in which the group occurs, as in consensus trees based on bootstrap analysis, but by the frequency of bipartitions in a set of weighted trees, termed a relative likelihood support score (Jermiin *et al.,* 1997). PROTML also provides for each branch a local bootstrap probability, which is the percentage of times the partition is supported when the four groups around the branch are considered.

For a parsimony analysis we used the concatenated data set and performed 1000 bootstrap simulations with TBR branchswapping and the MULPARS option enabled.

RESULTS

The DNA sequences have been deposited under GenBank Accession Numbers AF147041–47057 for the *CO1-CO2* region and U75354, U61490, and AF146712–46726 for *cytochrome b,* and the alignments inferred deposited with EMBL (Accession Numbers DS42559 and DS42560); the amino acid sequences used were inferred from the DNA sequences using the insect mitochondrial code. The tRNA gene sequences are shown in Fig. 1 folded into the inferred configurations of the corresponding tRNAs.

The base composition of the genes is biased, with simple mean AT% values of 79.7, 63.7, and 86.1 for the three codon positions in the *CO1* gene, 69.8, 70.9, and 86.4 for the *CO2* gene, and 70.5, 70.4, and 84.1 for the *cytochrome b* gene. Codon bias values calculated from the mean base compositions differed between codon positions and genes, being for the first position 0.4 for the *CO1* gene, 0.24 for *CO2,* and 0.28 for *cytochrome b,* with the values for the second position being 0.23, 0.28, and 0.30, and for the third position 0.48, 0.49, and 0.46.

The χ^2 test implemented in PUZZLE rejected each of the three codon positions in that three to nine sequences were found to differ significantly in composition from the average composition. In each case ingroup sequences were among those rejected. All protein sequences, however, were found not to differ significantly from the average composition. We therefore confined our attention to the protein sequences.

Comparisons between the four amino acid replacement models strongly supported the mtREV24 model over the others tested (Fig. 2).

Randomization tests on the concatenated amino acid sequences using RETICULATE indicate statistically significant differences in evolutionary rate between the three genes (Table 3). The observed compatibilities of the genes differ with *cytochrome b* having the lowest value and *cytochrome oxidase 1* having the highest value. Such a result can arise from differences in evolutionary rates (Jakobsen and Easteal, 1996), implying that *cytochrome b* evolved faster than *cytochrome oxidase 2* and that *cytochrome oxidase 1* evolved slower than *cytochrome oxidase 2.* This result corroborates those published previously [e.g., (Crozier and Crozier, 1993)].

Following the use of different starting trees and the rearrangement option of PROTML, a set of 23 trees was derived, comprising the most likely tree and 22 others not significantly worse than that according to the Kishino-Hasegawa test. The most likely tree is shown in Fig. 3C.

The morphology-based trees (Figs. 3A and 3B) were rejected at the 5% level as being significantly worse than the most likely tree in their fit to this data set.

The maximum-parsimony consensus tree is shown in Fig. 3D. It has various features in common with the ML tree and the ML consensus tree, and is not significantly worse than the ML tree according to the Kishino-Hasegawa test. However, it is characterized by generally low bootstrap support, with one of the best-supported branches contradicting subfamily placements among the outgroups.

The consensus tree derived using TREECONS and CONSENSE from the set of 23 trees under model V of Jermiin *et al.* (1997) is shown in Fig. 3E. This tree is not among the set of trees used to derive it, and is not significantly worse than the ML tree according to the Kishino-Hasegawa test. The consensus tree was used as a user tree in order to obtain local bootstrap probabilities and branch lengths. All branches with local bootstrap probabilities of 33% or less are not significantly different from zero. Addition of the MP topology to the set used to derive the ML consensus tree does not alter its topology and minimally affects support values.

DISCUSSION

tRNAs

In the ants reported here there is variation in many parts of the *tRNA* gene. The least variable section is the DHU arm, but although the DHU stem is invariant and the DHU loop is identical in most cases, in two cases there are insertions of one (*Liometopum*) or five (*Paratrechina*) nucleotides. The amino-acyl arm is also very similar throughout, save for an unpaired section at the 3′ end and a GC pair at the base in *Leptomyrmex.* The TΨC arm is variable both in size and in whether there is a GC pair in the stem (*Anonychomyrma, Iridomyrmex, Linepithema, Ochetellus, Papy-*

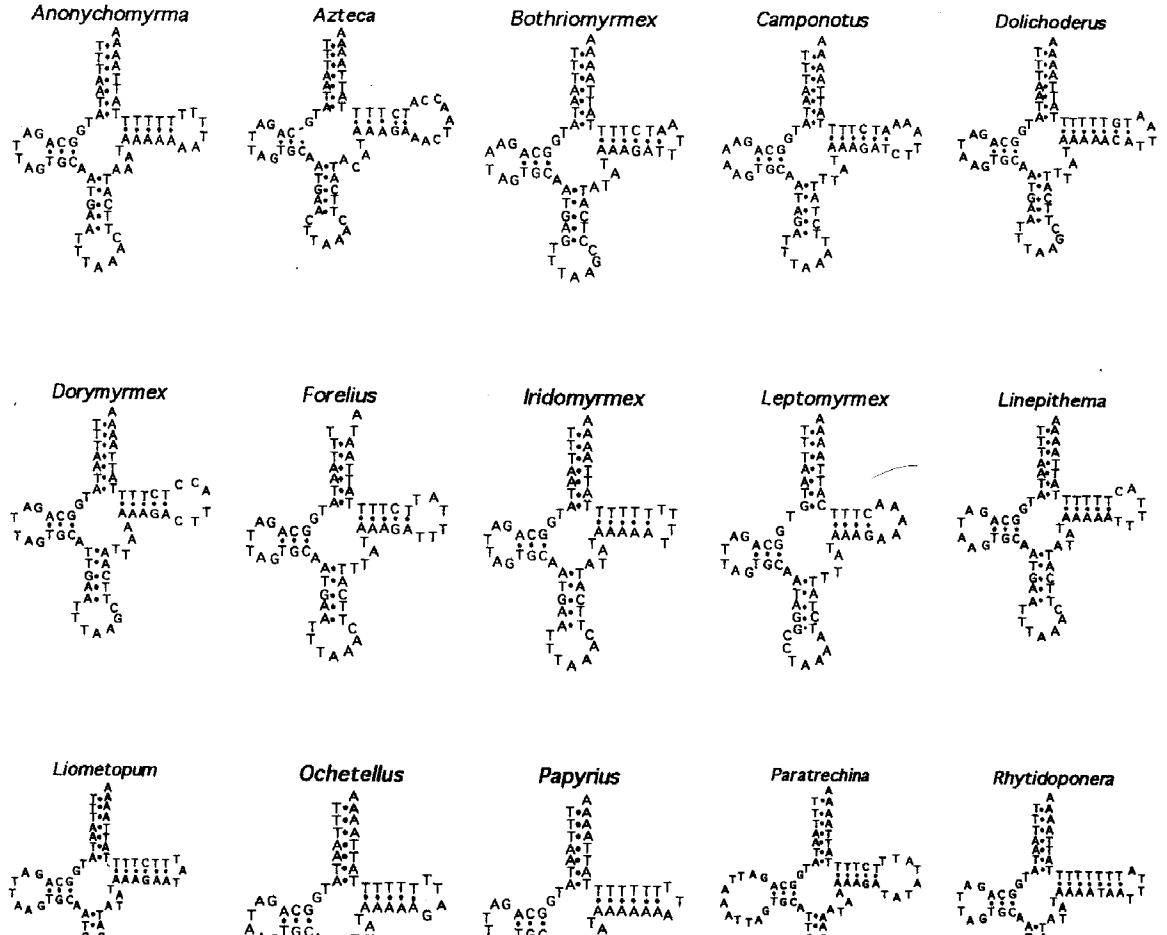

FIG. 1. *tRNA* gene sequences folded into the inferred structures of the tRNAs.

rius, and *Rhytidoponera* lack a TΨC GC pair). The anticodon arm is invariant in size and in the length of the stem, other than in *Paratrechina* in which the stem is 4 rather than 5 bp long (with incorporation of one pair into an enlarged loop). All sequences have one or more GC pairs in the anticodon stem. The base pair adjacent to the loop is a GT pair in *Leptomyrmex.*

Translation Termination

Mitochondrial genes often have abbreviated stop codons (T or TA) when followed immediately by a *tRNA* gene (Wolstenholme, 1992); in such cases, as in humans, complete termination codons result from polyadenylation following transcription (Ojala *et al.,* 1981)

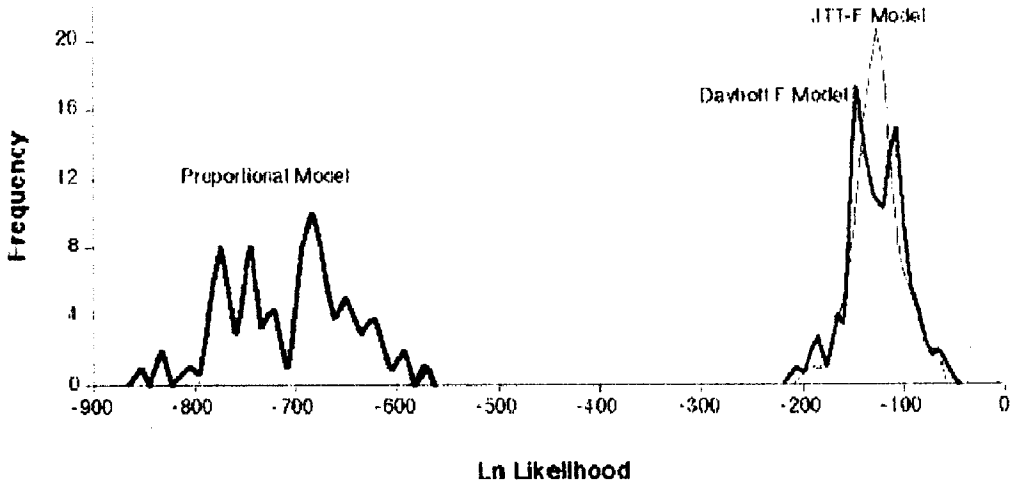

FIG. 2. Distributions of ln likelihoods derived from comparisons between the JTT, Proportional, and Dayhoff substitution models and the mtREV24 model, expressed as differences from the mtREV24 model, for the results of analyses using 100 pseudo-replicates of the protein sequence data.

and it is reasonable to suppose that this occurs in insects as well. The species presented here provide an unusual glimpse of evolutionary variation in translation termination: while the three outgroup taxa (*Camponotus, Paratrechina,* and *Rhytidoponera*) all present complete stop codons, all the dolichoderines present either a TA (*Bothriomyrmex, Liometopum, Tapinoma,* and *Technomyrmex*) or a T (all other species), as shown in Fig. 3.

In an extensive study of 15 families across all Hymenoptera, Dowton and Austin (1999) found many cases of apparent overlap between the *CO2* and the following *tRNA* genes, and between *tRNA* genes. The ant in their study was a species not showing overlap. Dowton and Austin (1999) note that, as in the *CO1-tRNA*$^{Leu}_{UUR}$ case of this paper, the *tRNA* genes following the *CO2* gene are transcribed from the opposite strand to that of the *CO2* gene, reducing the need to postulate polyadenylation and precise excision of the tRNA from the primary transcript. For the dolichoderines, precise excision of the tRNA with no overlap seems the more parsimonious model, given that without precise excision and polyadenylation some of the *CO1* genes would be considerably elongated (e.g., the *Bothriomyrmex CO1* gene would continue for all but the final two nt of the *tRNA*$^{Leu}_{UUR}$ gene). Under this interpretation, the lability of translation termination seen in this group indicates that a single T is probably always sufficient to terminate translation in insect mitochondrial genes when this co-occurs with an abutting *tRNA* gene.

TABLE 3

Randomization Tests Results Using RETICULATE

Gene	Observed compatibility	Shuffled average	Shuffled fraction exceeding observed
cytochrome oxidase 1	0.509971	0.383958	0.0371 +
cytochrome oxidase 2	0.441026	0.384393	0.0659 +
cytochrome b	0.313154	0.384278	0.0023 −

Translation Initiation

Only *Bothriomyrmex* and *Leptomyrmex* have a standard methionine initiation codon (ATA) for the *CO2* gene (Fig. 3); all other species have ATT or ATC, which are usually inferred to yield isoleucine. However from various lines of evidence it is possible (Wolstenholme, 1992) that when these codons occur as initiation codons they yield methionine.

Noncoding Regions

All three outgroup taxa present a stretch of noncoding sequence between the end of the *CO1* gene and the *tRNA*$^{Leu}_{UUR}$ gene, but none of the dolichoderines sampled show this feature, consistent with excision of the *tRNA*$^{Leu}_{UUR}$ from the polycistronic messenger facilitating the completion through polyadenylation of the stop codon (Ojala *et al.,* 1981). The occurrence of these noncoding stretches suggests that this region might provide variable markers in population studies of species of *Camponotus, Paratrechina,* and *Rhytidoponera.*

Phylogeny

There is considerable agreement between the MP and the ML consensus topologies, with both recognizing the grouping around *Iridomyrmex* and that of *Liometopum* + *Tapinoma* + *Technomyrmex,* possibly indicating that the data as used at least approximately meet the conditions for parsimony to yield the same result as likelihood (Felsenstein, 1983).

The most likely and consensus trees are very similar, with the main differences occurring as alternate reso-

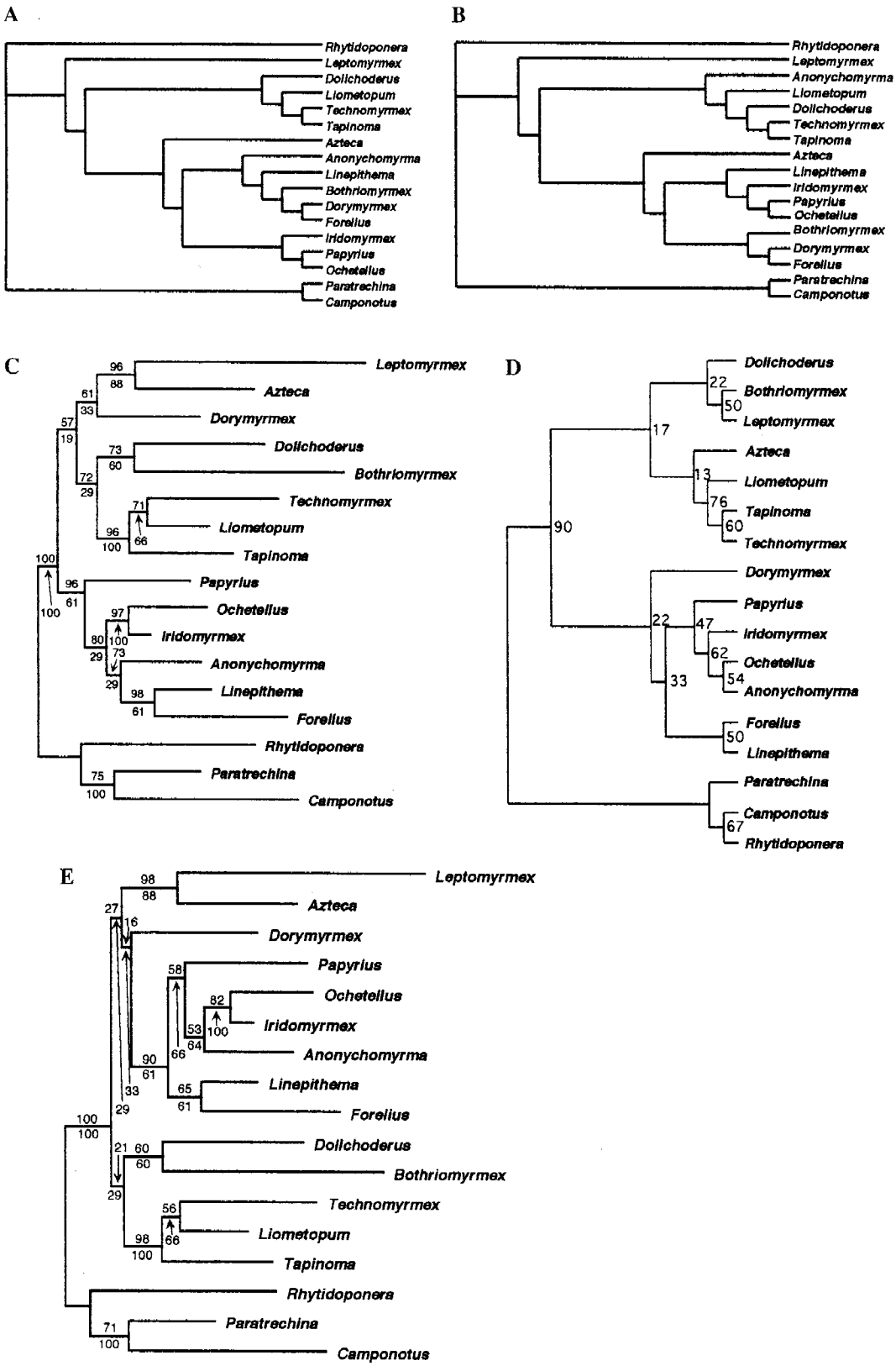

FIG. 3. Phylogenetic trees of the genera in this study, comprising 14 from the subfamily Dolichoderinae, one from the Ponerinae (*Rhytidoponera*), and two from the Formicinae (*Camponotus* and *Paratrechina*). (A) Genera arranged as in Shattuck's (1992a) Fig. 1, derived from a cladistic analysis of a 104-character data set in which 89 characters were ordered. (B) Arrangement as per Shattuck's (1992a) Fig. 2, derived from a majority-rule consensus tree of 70 equally parsimonious trees found when all characters were treated as unordered. (C) The maximum-likelihood tree based on the concatenated protein data set and the mtREV24 substitution model. Local bootstrap probabilities are shown above branches and relative likelihood support scores below them. (D). The maximum-parsimony consensus tree from 1000 bootstrap replicates, with percentage support values shown. (E) The maximum-likelihood consensus tree, labeled as for (C).

lutions of the nonsignificant branches in the consensus tree, and in the arrangement of genera in the cluster of six general including *Iridomyrmex.*

Particularly strongly supported groups are those of *Liometopum* + *Tapinoma* + *Technomyrmex* and *Anonychomyrma* + *Iridomyrmex* + *Ochetellus* + *Papyrius* + *Linepithema* + *Forelius.* The grouping of *Leptomyrmex* with *Azteca* is also quite strong, but the exceptionally long branch going to *Leptomyrmex* suggests that this placement may be anomalous. The *Bothriomyrmex* + *Dolichoderus* group is also quite well supported.

Various structural features of the tRNAs and the termination and initiation signals were not included in the quantitative analysis (the tRNA sequences were not part of the alignments used for the MP and ML analyses), and hence these qualitative features allow an independent check on the plausibility of the topologies. The placement together of *Anonychomyrma, Iridomyrmex, Linepithema, Ochetellus,* and *Papyrius* accords with the lack of a GC pair in the tRNA TΨC stem in all of these genera. This group clusters together the groups previously included in the genus *Iridomyrmex. Forelius* has a GC pair in the TΨC stem, and its membership of the *Iridomyrmex* group is therefore unexpected.

In terms of the hypotheses suggested by the morphological studies, the largest difference between the trees from the two kinds of data is the placement of *Leptomyrmex*—a sister-group to all other dolichoderines according to morphology but well within the cluster according to mtDNA. However, as noted above, the extreme length of the branch going to *Leptomyrmex* suggests that there may be features of its mtDNA evolution that yield anomalous placements. This may be a general feature affecting this genus—two of the three genes considered separately yielded the most likely tree, which includes this placement, and its $tRNA^{Leu}_{UUR}$ differs from those of other dolichoderines in two features of the anticodon stem.

The grouping of *Liometopum, Tapinoma,* and *Technomyrmex* suggested on morphological grounds is very strongly supported by the molecular data. The morphologically inferred group of these three genera plus *Dolichoderus* is broken up by the inclusion of *Bothriomyrmex,* but otherwise is at least weakly supported by the molecular analysis. The strong grouping of *Liometopum, Tapinoma,* and *Technomyrmex* shares with *Bothriomyrmex* the possession of TA stop codons differentiating them from all other dolichoderines sampled.

The morphological group of *Bothriomyrmex* + *Dorymyrmex* + *Forelius* draws no support from the molecular data.

Considering that many of the deeper nodes in the consensus tree are weakly supported, other disagreements between the molecular and the morphological results should not be strongly emphasized. Overall, there are many tantalizing points of agreement, as well as disagreement, between the trees derived from the two kinds of data.

Given that extensive local rearrangements did not improve any of the set of 23 trees, it is likely that there are many other yet undiscovered local optima in the likelihood surface for these data, a situation which would not have been revealed without extensive use of different seed trees in the analysis.

ACKNOWLEDGMENTS

We thank D. R. Wolstenholme for discussions on *tRNA* genes, A. T. Beckenbach for allowing us to use some of his unpublished primers, John W. Wenzel and an anonymous referee for comments on the manuscript, and P. S. Ward and especially S. O. Shattuck for specimens, identifications, and phylogenetic discussions during the course of this work. This work was supported by Australian Research Council grants to R.H.C. and was in part included in a thesis forming part of the requirements for an honors degree from La Trobe University for M.C. The School of Biological Sciences at the University of Sydney is gratefully acknowledged for providing temporary space and resources to L.S.J.

REFERENCES

Adachi, J., and Hasegawa, M. (1996a). Model of amino acid substitution in proteins encoded by mitochondrial DNA. *J. Mol. Evol.* **42:** 459–468.

Adachi, J., and Hasegawa, M. (1996b). MOLPHY 2.3. Programs for Molecular Phylogenetics Based on Maximum Likelihood, Institute of Statistical Mathematics, Tokyo.

Ayala, F. J., Wetterer, J. K., Longino, J. T., and Hartl, D. L. (1996). Molecular phylogeny of *Azteca* ants (Hymenoptera: Formicidae) and the colonization of *Cecropia* trees. *Mol. Phylogenet. Evol.* **5:** 423–428.

Baroni Urbani, C., Bolton, B., and Ward, P. S. (1992). The internal phylogeny of ants (Hymenoptera: Formicidae). *Syst. Entomol.* **17:** 301–329.

Baur, A., Buschinger, A., and Zimmermann, F. K. (1993). Molecular cloning and sequencing of 18S rDNA gene fragments from six different ant species. *Insect. Soc.* **40:** 325–335.

Baur, A., Chalwatzis, N., Buschinger, A., and Zimmermann, F. K. (1995). Mitochondrial DNA sequences reveal close relationships between social parasitic ants and their host species. *Curr. Genet.* **28:** 242–247.

Baur, A., Sanetra, M., Chalwatzis, N., Buschinger, A., and Zimmermann, F. K. (1996). Sequence comparisons of the internal transcribed spacer region of ribosomal genes support close relationships between parasitic ants and their respective host species (Hymenoptera: Formicidae). *Insect. Soc.* **43:** 53–67.

Bolton, B. (1995). "A New General Catalogue of the Ants of the World" Harvard Univ. Press, Cambridge, MA.

Bourke, A. F. G., and Franks, N. R. (1995). "Social Evolution in Ants" Princeton Univ. Press, Princeton, NJ.

Brown, W. L. (1973). *In* "Tropical Forest Ecosystems in Africa and South America: A Comparative Review" (B. J. Meggers, E. S. Ayens, and W. D. Duckworth, Eds.), pp. 161–185, Smithsonian Inst. Press, Washington, DC.

Crozier, R. H., and Crozier, Y. C. (1993). The mitochondrial genome of the honeybee *Apis mellifera:* Complete sequence and genome organization. *Genetics* **133:** 97–117.

Crozier, R. H., Dobric, N., Imai, H. T., Graur, D., Cornuet, J.-M., and Taylor, R. W. (1995). Mitochondrial-DNA sequence evidence on the phylogeny of Australian Jack-jumper ants of the *Myrmecia pilosula* complex. *Mol. Phylogenet. Evol.* **4:** 20–30.

Crozier, R. H., Jermiin, L. S., and Chiotis, M. (1997). Molecular evidence for a Jurassic origin of ants. *Naturwissenschaften* **84:** 22–23.

Crozier, R. H., and Pamilo, P. (1996). "Evolution of Social Insect Colonies. Sex Allocation and Kin-Selection" Oxford Univ. Press, Oxford, UK.

Dayhoff, M. O., Schwartz, R. M., and Orcutt, B. C. (1978). *In* "Atlas of Protein Sequence and Structure" (M. O. Dayhoff, Ed.), Vol. 5, Suppl. 3, pp. 345–352, National Biomed. Res. Found., Washington, DC.

Dowton, M., and Austin, A. D. (1999). Evolutionary dynamics of a mitochondrial rearrangement "hot spot" in the Hymenoptera. *Mol. Biol. Evol.* **16:** 298–309.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* **17:** 368–376.

Felsenstein, J. (1983). Parsimony in systematics: Biological and statistical issues. *Annu. Rev. Ecol. Syst.* **14:** 313–333.

Hölldobler, B., and Wilson, E. O. (1990). "The Ants" Harvard Univ. Press, Cambridge, MA.

Jakobsen, I. B., and Easteal, S. (1996). A program for calculating and displaying compatibility matrices as an aid in determining reticulate evolution in molecular sequences. *Comp. Appl. Biosci.* **12:** 291–295.

Jermiin, L. S., Olsen, G. J., Mengersen, K. L., and Easteal, S. (1997). Majority-rule consensus of phylogenetic trees obtained by maximum-likelihood analysis. *Mol. Biol. Evol.* **14:** 1296–1302.

Jones, D. T., Taylor, W. R., and Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Comput. Appl. Biosci.* **8:** 275–282.

Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* **29:** 170–179.

Lewontin, R. C., and Felsenstein, J. (1965). The robustness of homogeneity tests in $2 \times n$ tables. *Biometrics* **21:** 19–33.

Lockhart, P. J., Steel, M. A., Hendy, M. D., and Penny, D. (1994). Recovering evolutionary trees under a more realistic model of sequence evolution. *Mol. Biol. Evol.* **11:** 605–612.

Lowe, T., and Eddy, S. (1997). tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25:** 955–964.

Ojala, D., Montoya, J., and Attardi, G. (1981). tRNA punctuation model of RNA processing in human mitochondria. *Nature* **290:** 470–474.

Shattuck, S. O. (1992a). Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). *Sociobiology* **21:** 1–181.

Shattuck, S. O. (1992b). Higher classification of the ant subfamilies Aneuretinae, Dolichoderinae and Formicinae (Hymenoptera: Formicidae). *Syst. Entomol.* **17:** 199–206.

Shattuck, S. O. (1992c). Review of the dolichoderine ant genus *Iridomyrmex* Mayr with descriptions of three new genera (Hymenoptera: Formicidae). *J. Aust. Entomol. Soc.* **31:** 13–18.

Shattuck, S. O. (1995). Generic-level relationships within the ant subfamily Dolichoderinae (Hymenoptera, Formicidae). *Syst. Entomol.* **20:** 217–228.

Strimmer, K., Goldman, N., and von Haeseler, A. (1997). Bayesian probabilities and quartet puzzling. *Mol. Biol. Evol.* **14:** 210–211.

Strimmer, K., and von Haeseler, A. (1996). Quartet puzzling: A quartet maximum-likelihood method for reconstructing tree topologies. *Mol. Biol. Evol.* **13:** 964–969.

Swofford, D. L. (1993). "Phylogenetic Analysis Using Parsimony (PAUP)" Illinois Natural History Survey, Champaign, IL.

Tay, W. T., Cook, J. M., Rowe, D. J., and Crozier, R. H. (1997). Migration between nests in the Australian arid-zone ant *Rhytidoponera* sp. 12 revealed by DGGE analyses of mitochondrial DNA. *Mol. Ecol.* **6:** 403–411.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25:** 4876–4882.

Tsuji, K., Furukawa, T., Kinomura, K., Takamine, H., and Yamauchi, K. (1991). The caste system of the dolichoderine ant *Technomyrmex albipes* (Hymenoptera: Formicidae): Morphological description of queens, workers and reproductively active intercastes. *Insect. Soc.* **38:** 413–422.

Wetterer, J. K., Schultz, T. R., and Meier, R. (1998). Phylogeny of fungus-growing ants (tribe Attini) based on mtDNA sequence and morphology. *Mol. Phylogenet. Evol.* **9:** 42–47.

Wilson, E. O. (1992) *In* "Biology and Evolution of Social Insects" (J. Billen, Ed.), pp. 1–7, Leuven, Leuven, Belgium.

Wolstenholme, D. R. (1992). Animal mitochondrial DNA: Structure and evolution. *Int. Rev. Cytol.* **141:** 173–216.