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Phylogenetic Relationships of North American Field Crickets Inferred from Mitochondrial DNA Data

Yuan Huang, Guillermo Ortí, Marie Sutherlin, Amy Duhachek, and Anthony Zera[1]

School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588

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A well-supported molecular phylogeny for North American *Gryllus* species based on a combined data set of mitochondrial (mt) DNA is presented. A total of 26 individuals representing 13 populations of 11 species of the genus *Gryllus* and 4 individuals of two outgroup species, *Teleogryllus oceanicus* and *Acheta domestica,* were sampled in this study. The complete cytochrome *b* gene (1036 bp) and a 500-bp fragment of the 16S rRNA gene were sequenced for each individual. Since results from separate analyses of the cytochrome *b* and 16S data sets, as well as a previously published mtDNA restriction-site data set, were not conflicting, all data were combined for phylogenetic analyses. The clade of European *Gryllus* was clearly separated from the North American clade. The amount of sequence divergence between these clades was significantly greater than within the clades, suggesting a basal drift-vicariant event in the genus. This is the first phylogenetic analysis of North American *Gryllus* that includes western species. Four well-supported groups were identified but their relationships showed no clear east-west structure. Our phylogeny supports the recent reassignment of *G. integer* Scudder 1901 from Texas to *G. texensis* Cade and Otte 2000. The evolution of cricket song and life cycle is discussed using the new phylogenetic framework.

INTRODUCTION

Species of North American crickets of the genus *Gryllus* have been used as models to investigate many important topics in evolutionary biology. These include the evolution of life cycles (Alexander, 1962, 1968; Masaki and Walker, 1987; Harrison and Bogdanowicz, 1995), the evolution of acoustical communication (Alexander, 1962, 1968; Walker, 1974; Libersat *et al.*, 1994), mechanisms of speciation (Fulton, 1952; Alexander and Bigelow, 1960; Alexander, 1968; Harrison, 1978; Harrison and Rand, 1989; Otte, 1989), the evolution of flightlessness (Zera and Denno, 1997), and the evolution of physiology (Zera and Zhang, 1995; Zera and Denno, 1997; Zera and Huang, 1999). Several of these studies involve interspecific comparisons and hence require a well-supported phylogeny as a framework to investigate character evolution (Harvey and Pagel, 1991; Brooks and McLennan, 1991). Despite the widespread use of species of *Gryllus* in interspecific evolutionary studies, there currently is no well-resolved phylogeny of this group.

Nine distinct species of *Gryllus* have been named in Eastern North America using behavioral (song) and ecological (life cycle) characteristics (Fulton, 1952; Bigelow, 1958; Alexander and Bigelow, 1960; Alexander and Walker, 1962; Walker, 1974; Cade and Otte, 2000). Nine species also have been named in California and Baja California, Mexico, and three are thought to be conspecific with Eastern North American *Gryllus* (Weissman *et al.*, 1980). Alexander (1968) derived a "probable phylogeny" of eastern *Gryllus* species based on a phenetic analysis of a limited number of behavioral and ecological characters. This phylogeny was used to infer the pattern and mode of evolution of life cycle (i.e., developmental stage at which diapause occurs), calling song, and habitat associations. Alexander and Bigelow (1960) proposed that a novel mode of speciation, allochronic speciation, accounted for the evolution of life cycles of some *Gryllus* species in Eastern North America. Subsequent phylogenetic analyses of this group, based on allozymes (Harrison, 1979), or mitochondrial (mt) restriction fragment length polymorphisms (Harrison and Bogdanowicz, 1995), indicated a very different pattern of life cycle evolution that was inconsistent with Alexander's hypothesis of allochronic speciation.

Although a major improvement over Alexander's early hypothesis, the analysis of Eastern North American *Gryllus* by Harrison and Bogdanowicz (1995) has left many important phylogenetic issues unresolved. One of the major shortcomings was that described species of *Gryllus* from Western North America were not investigated. Although Harrison and Bogdanowicz

[1] To whom correspondence should be addressed at School of Biological Sciences, 348 Manter Hall, University of Nebraska, Lincoln, NE 68588-0118. Fax: (402) 472-2083. E-mail: AZERA@unlserve.edu.

1055-7903/00 $35.00

TABLE 1

***Gryllus* Species and Outgroup Taxa Included in This Study**

Species	Collection locality	GenBank Accession[a]
G. assimilis	Homestead, Florida	AF248657-8, AF248684
G. assimilis	Santa Barbara, California	AF248655-6, AF248683
G. bimaculatus	Cyprus	AF248659-60, AF248685
G. campestris	Germany	AF248661-2, AF248686
G. firmus	Gainesville, Florida	AF248663-4, AF248687
G. integer	Davis, California	AF248667-8, AF248689
G. texensis	Austin, Texas	AF248669-70, AF248690
G. fultoni	Gainesville, Florida	AF248665-6, AF248688
G. lineaticeps	Santa Barbara, California	AF248671-2, AF248691
G. ovisopis	Gainesville, Florida	AF248673-4, AF248692
G. rubens	Gainesville, Florida	AF248676-7, AF248694
G. veletis	Sharon, Connecticut	AF248678-9, AF248695-6
G. pennsylvanicus	Ithaca, New York	AF248675, AF248693
Teleogryllus Oceanicus	Australia	AF248680-1, AF248697
Acheta domesticus	Unknown[b]	AF248682, AF248698

[a] Cytochrome *b* and 16S fragment, respectively.
[b] Purchased from Carolina Biological Supply Co. (U.S.A.).

(1995) provided consistent evidence for two clades of mainly Eastern North American *Gryllus*, the relationship of these clades with the other species included in their study remained unclear. Furthermore, their analysis suggested that the European clade (represented by *G. bimaculatus* and *G. campestris*) was nested among the North American species. Based on restriction-site data, the estimated mtDNA sequence divergence between crickets in different continents was comparable to divergences among North American species. This evidence led Harrison and Bogdanowicz (1995) to postulate recent migration of crickets between continents either by dispersal across a Bering land bridge or by long-distance flight across the Atlantic Ocean.

The lack of a well-resolved phylogeny of North American *Gryllus* species has precluded rigorous comparative studies of physiological characteristics in species of this genus (Zera *et al.*, 1998; Zera and Huang, 1999) and prompted us to investigate further the phylogeny of this group. Here we report on a phylogenetic analysis based on DNA sequences of two mitochondrial genes, the complete cytochrome *b* and a fragment of the 16S rRNA gene. The goals of our study were to better resolve the phylogenetic relationships among North American *Gryllus* species, and between North American and European species. We also investigated the phylogenetic relationships between several Eastern and Western North American *Gryllus* species. Two of these species, *G. assimilis* and *G. integer*, have been the foci of a number of evolutionary studies (Zera and Zhang, 1995; Zera *et al.*, 1998; Smith and Cade, 1987; Cade and Tyshenko, 1990; Walker, 1998; Cade and Otte, 2000). Finally, we used our phylogeny, in combination with the phylogeny of Harrison and Bogdanowicz (1995), to study the evolution of life cycles and song in *Gryllus*.

MATERIALS AND METHODS

Taxon Sampling

A total of 26 individuals representing 13 populations of 11 species of the genus *Gryllus* and 4 individuals of two outgroup species, *Teleogryllus oceanicus* and *Acheta domestica*, were sampled in this study. Two individuals from each population were assayed to gauge intraspecific variation and to corroborate the accuracy of our results. Detailed information on the specimens is listed in Table 1.

Laboratory Procedures

Total cellular DNA was extracted from cephalic tissues only from individual specimens by the standard proteinase K and phenol/chloroform extraction method (Sambrook *et al.*, 1989). Fresh, frozen, or 95% ethanol-preserved specimens usually gave the same quality and quantity of DNA. Three overlapping fragments of the cytochrome *b* gene (total of 1036 bp) and a 550-bp fragment of the 16S rRNA gene were amplified via the polymerase chain reaction (PCR) from each individual DNA sample. The primers used are shown in Table 2. PCRs were done in 25-μl volumes with a final concentration of 0.8 μM of each primer, 1 mM of each dNTP, 2–4 mM $MgCl_2$, and 0.5 units of *Taq* polymerase (Gibco BRL). After checking PCR products on 1.5% agarose gels, they were purified using Wizard PCR Preps (Promega) and directly sequenced (in both directions) using the BigDye terminator ready-reaction kit and resolved on either ABI 377 or 310 Genetic Analyzers (PE Ap-

TABLE 2

Primers Used for PCR Amplification and Sequencing

Name	Sequence (5′–3′)[a]	Source
P21	CCATCCAACATCTCAGCATGATGAAA	Kocher *et al.* (1989)
P22	GCCCCTCAGAATGATATTTGTCCTCA	Kocher *et al.* (1989)
2F	GTAATAGCAACAGCWTTTATAG	This study
2F2	GTTATAGCTGCAGCCTTTATTG	This study
2R	CCWARTTTATTAGGAATAGATCG	This study
3F	CCAGCWAAYCCYTTAGTAAC	This study
3F2	TAGGAGATCCAGATAATTTTAC	This study
3R	GCTTWKTYAAGCTMATTAACTTA	This study
16Sa	CGCCTGTTTAACAAAAACAT	Kocher *et al.* (1989)
16Sb	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)

[a] Degenerate sites are indicated by W = A or T; Y = C or T; K = G or T; M = A or C.

TABLE 3

Number of Variable and Parsimony-Informative Sites among Cricket Cytochrome *b* Sequences[a]

Categories (total bp)	No. of variable sites (% of total in category)	No. of informative sites (% of total in category)	PTP[b]
All positions (1036 bp)	357 (34.5%)	215 (20.7%)	0.0002
First codon positions (345 bp)	76 (22%)	36 (10.4%)	0.0002
Second codon positions (345 bp)	37 (10.7%)	13 (3.8%)	0.0446
Third codon positions (345 bp)	243 (70.4%)	166 (48.1%)	0.0002
Protein (345 aa)	74 (21.5%)	21 (6.1%)	0.0002

[a] Only one sequence per species was used.
[b] Probability associated with the test for significant phylogenetic structure.

plied Biosystems), following the manufacturer's recommendations. Sequence data were edited and compiled using Sequencher version 3.0 (Gene Codes Corp.). All sequences used in this study have been deposited in GenBank (see Table 1 for accession numbers).

Phylogenetic Analysis

For the 16S rRNA fragment, DNA sequences were aligned using CLUSTALW 1.5 (Thompson *et al.*, 1994) with default settings (opening gap cost = 20, extending gap cost = 5). Cytochrome *b* sequences were assembled, aligned, and translated with Sequencher 3.1 (Gene Codes Corp.). Two approaches were used to gauge phylogenetic signal in the DNA sequence data sets. Potential saturation of transitions at third codon positions in the cytochrome *b* gene was assessed by comparing the number of changes at these positions as a function of sequence divergence (Fig. 1). Sequence statistics were obtained using the program MEGA version 1.0 (Kumar *et al.*, 1993). Phylogenetic structure in the data also was assessed using the PTP test (Faith, 1991) as implemented in PAUP*, with 5000 random matrices and randomizing ingroup taxa only (using a single sequence per species). This procedure tests if a data set has any structure that is significantly different from random.

Phylogenetic analyses under the maximum-parsimony (MP), maximum-likelihood (ML), and minimum-evolution (ME; Kidd and Sgaramella-Zonta, 1971; Rzhetsky and Nei, 1992) criteria were performed as implemented in PAUP* version 4.0 b (Swofford, 1998). Heuristic searches were conducted using stepwise addition with 100 random replications. The appropriate model of DNA substitution for ML and ME analyses was determined using MODELTEST version 3 (Posada and Crandall, 1998). This procedure implements a hierarchical likelihood ratio test to determine the model that best fits the data. Parameters for the chosen model (base composition, substitution rates, proportion of invariable sites, and gamma shape parameter) were estimated by maximum likelihood on a neighbor-joining (NJ) tree (Saitou and Nei, 1987).

Following Bull *et al.* (1993), the different data partitions (cytochrome *b*, 16S, and restriction sites) were tested for heterogeneity before being combined for further analysis. We assessed heterogeneity using the partition homogeneity test developed by Farris *et al.* (1994, 1995) and implemented in PAUP*. The test is based on the observed incongruence tree length difference resulting from combining the data matrices. The observed tree length difference is tested for significance against a null distribution generated by randomly partitioning the pooled data matrices. Partition-homogeneity tests were performed with 1000 iterations for each test.

Nonparametric bootstrapping (Felsenstein, 1985; Hillis and Bull, 1993), based on 1000 replications, was used to assess support for the resulting topologies. Relative rate tests, based on the two-cluster test and the branch length tests of Takezaki *et al.* (1995), implemented in their software package LINTRE, were used to test for rate constancy among lineages in the resulting phylogeny (i.e., the molecular clock assumption). This procedure also constructs a linearized tree that can be used to estimate divergence values among clades under the assumption of rate constancy.

The best-supported phylogenetic hypothesis was used to investigate the evolution of life history traits. Most-parsimonious reconstructions of life history characters obtained from the literature were optimized with MacClade version 3.07 (Maddison and Maddison, 1992). Calling songs were coded as described by Alexander (1962) and life cycle characters (i.e., diapause stage) for most species were taken from Harrison and Bogdanowicz (1995).

RESULTS

The complete cytochrome *b* gene (a total of 1036 bp) was sequenced for each individual. Among all species considered, 357 sites are variable, and 215 are parsimony informative (see Table 3). Significant phylogenetic structure was detected with the PTP test for all except second codon positions and for the translated protein sequence ($P = 0.0002$, Table 3). More than

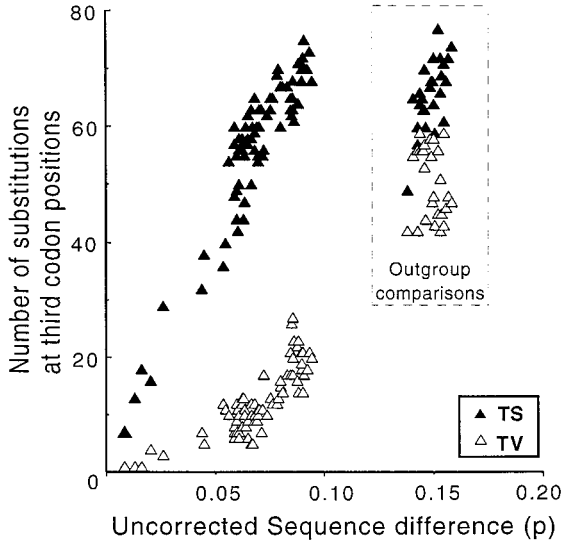

FIG. 1. Substitution pattern at third codon positions of the cytochrome *b* gene. The number of transition (TS) and transversion (TV) substitutions at third codon positions is plotted against uncorrected sequence divergence (*p*) considering all sites. Each point represents a pairwise comparison among species. Comparisons involving outgroup taxa are highlighted to show TS saturation.

77% of all informative sites are third codon position substitutions. To assess the pattern of variation at third codon positions, the number of transitions (TS) and transversions (TV) were plotted against the uncorrected distance "*p*" (proportion of differences at all sites among two sequences), for all pairwise comparisons (Fig. 1). This analysis showed that, at third codon positions in the cytochrome *b* gene, TS and TV substitutions *within* ingroup taxa are not saturated (there is a positive slope in the graph) but that TS substitutions between the *Gryllus* species and the outgroup may be saturated (the number of TS reaches a plateau between 60 and 70 changes). On the other hand, TV substitutions do not appear to be saturated since the number of transversions increases monotonically with sequence divergence (Fig. 1). The transition/transversion ratio (estimated by maximum likelihood on the MP tree shown in Fig. 2) is TS/TV = 4.8. But this ratio estimated for third codon positions only is TS/TV = 8.9. Therefore, for the cytochrome *b* data set, we use variation at all positions for phylogenetic inference but also test for the effects of saturation at third codon transitions using weighted parsimony and a justified model of sequence evolution (determined by MODELTEST).

Total alignment length for the 16S rRNA sequences is 500 bp (obtained using Clustal W). Partition-homogeneity tests did not reject the null hypothesis that the cytochrome *b* and 16S data sets are any different from random partitions of the pooled data. We tested the following partitions: codon positions within cytochrome *b* ($P = 0.97$) and cytochrome *b* versus the 16S ($P = 0.84$). Therefore, both data sets were combined (cytochrome *b* and 16S) and used for all subsequent phylogenetic analyses. Results from separate phylogenetic analyses are not conflicting and are not presented here for simplicity. The combined data set has a total of 1536 sites, 454 sites of which are variable and 271 sites parsimony-informative (when a single sequence per species is used). Significant phylogenetic structure was detected by the PTP test ($P = 0.0002$). The general time reversible model with among-site rate heterogeneity (GTR + Γ; Lanave *et al.*, 1984; Yang, 1994; Gu *et al.*, 1995) was selected by MODELTEST as the best fit for the combined data set. Rate matrix parameters estimated on the NJ tree are $R(a) = 2.44$, $R(b) = 8.36$, $R(c) = 3.08$, $R(d) = 1.27$, $R(e) = 37.89$, $R(f) = 1.00$. *Base frequencies are* $A = 0.32$, $C = 0.16$, $G = 0.15$, $T = 0.36$. Among-site rate variation was approximated with the gamma distribution shape parameter $a = 0.19$. These parameters were used for subsequent phylogenetic analyses on the combined data set.

Phylogenetic Analysis

A single most-parsimonious tree (length = 910, CI excluding uninformative characters = 0.596, retention index = 0.82) was obtained with the combined DNA sequence data set under unweighted parsimony, treating gaps as missing information (Fig. 2). The same tree (but three steps longer) was obtained when gap character states were treated as a "fifth base." The effect of transitions at third codon positions of the cytochrome *b* gene was tested by weighted parsimony. A single most-parsimonious tree was obtained when transitions at these positions were down-weighted by a factor of 2 or 5 with respect to all other substitutions (Fig. 3). The same tree was obtained under ME using the GTR + Γ model described above. Bootstrap values for weighted parsimony and ME (shown in Fig. 3) suggest a strong support for this topology (Hillis and Bull, 1993). ML analysis resulted in the same tree, except that *G. assimilis* was placed as the sister taxon of *G. fultoni* (with bootstrap support <50). Parsimony analysis of the cytochrome *b* protein sequence (345 characters), using the "protpars" stepmatrix, resulted in a topology very similar to Fig. 3. Again, the main difference was the placement of *G. assimilis,* now as the sister species to *G. integer* (from CA), and the placement of *G. veletis* as the basal taxon for the group 3 + 4 shown in Fig. 3.

The mtDNA restriction-site data set published by Harrison and Bogdanowicz (1995) was tested for homogeneity against the combined mtDNA sequence data. The null hypothesis that these two data partitions are homogeneous was not rejected ($P = 1.00$); thus we proceeded to combine all the data for a final analysis. Weighted parsimony (down-weighting the third posi-

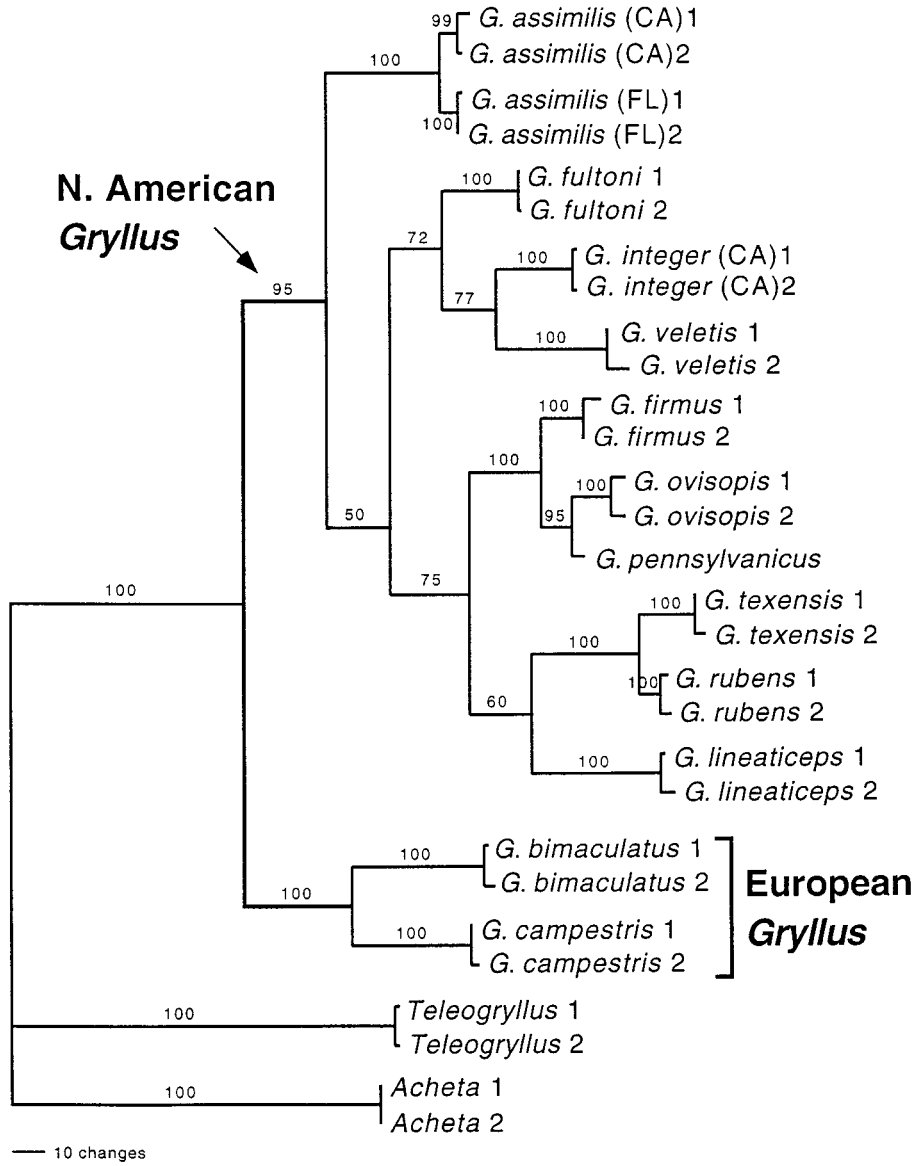

FIG. 2. Most-parsimonious tree obtained with unweighted parsimony on the combined cytochrome *b* and 16S data sets (1536 bp). Tree length is 910 steps (alignment gaps were treated as "missing"), the consistency index (excluding uninformative characters) is CI = 0.596, and the retention index = 0.82. Bootstrap support values, calculated with 1000 replications, are shown for each branch. Two specimens from each locality are included in the analysis, except for *G. pennsylvanicus*.

tion transitions 1:5) and assigning equal weights to restriction-site variation and all other DNA substitutions resulted in the same tree as shown in Fig. 3. The same result was obtained treating restriction-site variation under a relaxed Dollo criterion (Swofford *et al.*, 1996), using up to a 7/1 ratio for gain/loss for each site. Therefore, based on the total available mtDNA information, we propose the topology shown in Fig. 3 as the preferred working hypothesis for this group. Life cycle and calling song characteristics were mapped onto the topology shown in Fig. 3. The results of our phylogenetic analysis are shown in Figs. 4 and 5.

Genetic Divergence

A nonsignificant value (Q = 9.22, 13 degrees of freedom) was obtained for the molecular clock test that combines the rate difference for all interior nodes under the root (two-cluster test) and for the branch-length test (root-to-tip distances) for each sequence (Takezaki *et al.*, 1995). This means that the data set does not depart significantly from molecular clock assumptions. A linearized tree (Takezaki *et al.*, 1995) was constructed with all taxa following the topology shown in Fig. 3 to estimate genetic divergence among

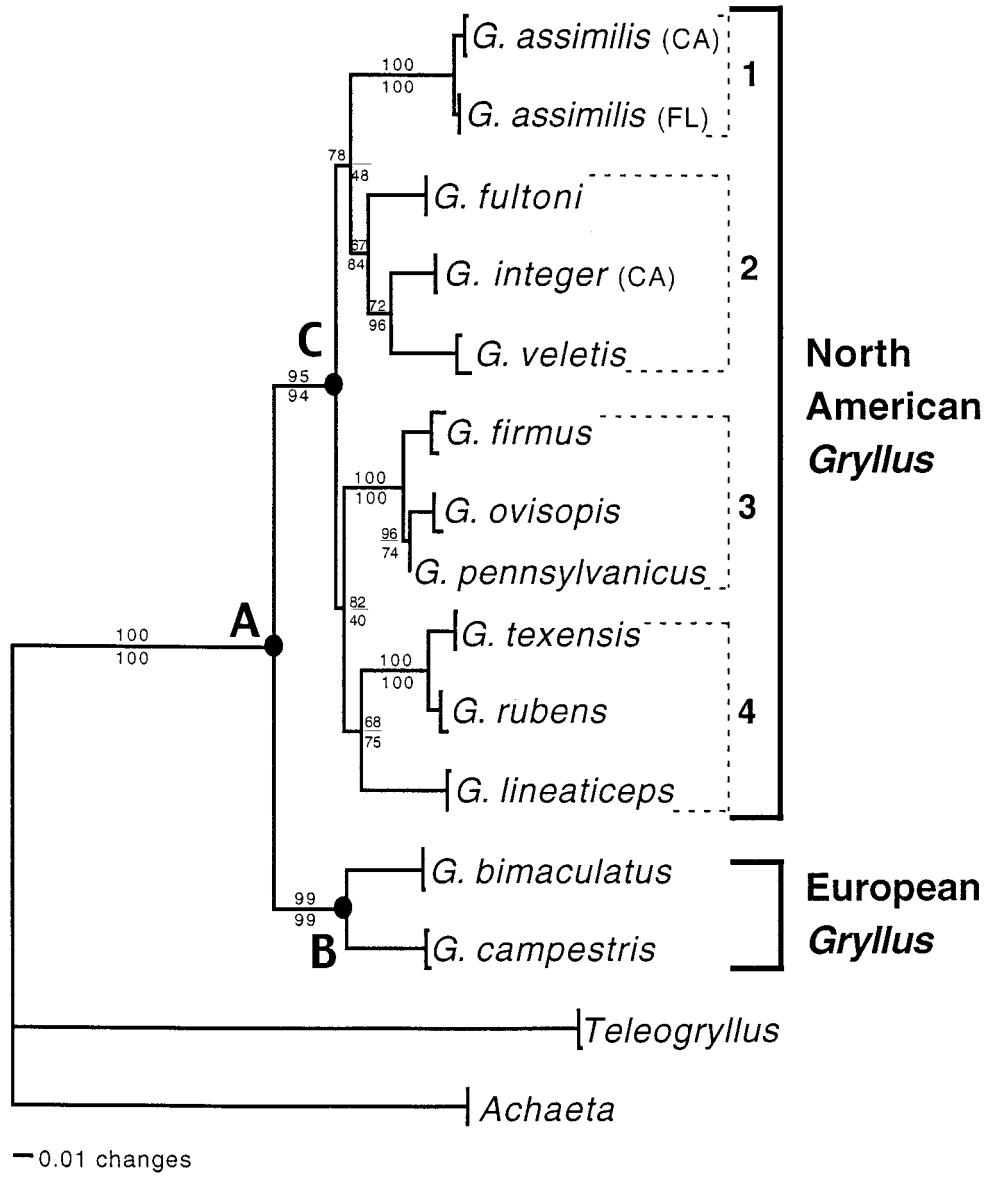

FIG. 3. Minimum evolution tree for the combined cytochrome *b* and 16S data sets (1536 bp) under the GTR + Γ model (see results section for parameter values). The same topology was obtained under weighted parsimony (down-weighting transitions at third codon positions). Bootstrap values for weighted parsimony (with transition:transversion changes at third codon positions weighted 1:5) and for ME analysis are shown above and below each branch, respectively. Only a single label was used to represent each population/species instead of two as shown in Fig. 2. Important nodes and North American clades discussed in the text are labeled A–C and numbered 1–4, respectively.

clades. The substitution model TrN + Γ (Tamura and Nei, 1993) was used to estimate genetic divergence since it was the closest alternative in LINTRE to the GTR + Γ model chosen by MODELTEST. Genetic divergence values are given as heights of each node of interest and represent the distance (± standard error) from that node to the tips, under the molecular clock assumption. Divergence between European and North American *Gryllus* in this tree (height of node A in Fig. 3) was 0.101 ± 0.004. Divergence among European species (height of node B in Fig. 3) was 0.052 ± 0.010, and the largest divergence among the North American species (height of node C in Fig. 3) was 0.068 ± 0.002. Clearly, the separation of European and North American lineages of *Gryllus* predated the speciation events in each continent.

DISCUSSION

Data Analysis

Cytochrome *b* and 16S sequences show substantial variation and similar levels of divergence among taxa. Although 16S is less variable overall, most (>77%) of

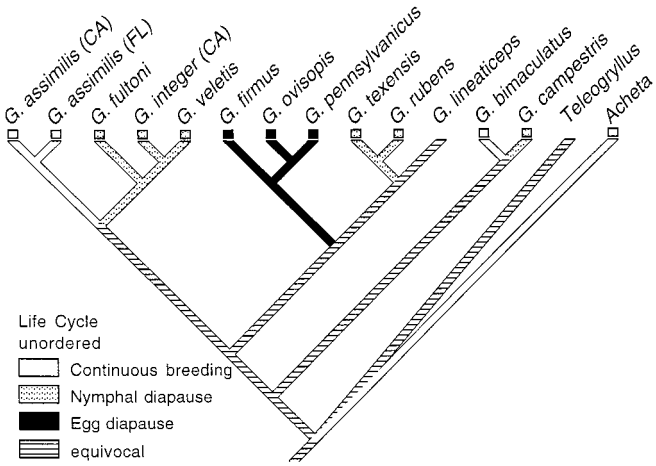

FIG. 4. Evolution of life cycle. The most-parsimonious reconstruction of life-cycle characters is based on the tree presented in Fig. 3.

the variation in cytochrome *b* was observed at third codon positions. Both data sets exhibit significant (nonrandom) phylogenetic structure, as suggested by PTP tests and phylogenetic signal was not conflicting among data partitions (including the restriction-site data). This is not surprising, since cytochrome *b*, 16S, and the restriction-site data all represent a single (mitochondrial) genetic unit not expected to undergo recombination. More significantly, consistency of tree selection among data partitions also suggests that the models used to analyze these data are adequate and recover a unique historical signal (Miyamoto *et al.*, 1995). However, the issue of whether to combine data sets in phylogenetic analysis remains controversial and an apparent consensus has yet to emerge when conflict among data partitions is significant (e.g., Kluge, 1989; Bull *et al.*, 1993; Chippindale and Wiens, 1994; de Queiroz *et al.*, 1995; Miyamoto and Fitch, 1995; Huelsenbeck *et al.*, 1996). But given that none of the partition homogeneity tests was significant, combination of all data for a single analysis is expected to maximize the amount of information and will most likely yield the correct tree (e.g., Vogler and Welsh, 1997; Chippindale *et al.*, 1999). The total information contained in the mitochondrial DNA data provides a robust phylogenetic hypothesis for this set of taxa. Our results strongly support earlier results (Harrison and Bogdanowicz, 1995) and provide further resolution to poorly resolved clades (see below).

We implemented a diversity of phylogenetic inference methods to the combined molecular data. We employed two different approaches for parsimony: equal weighting of all substitution types and differential weighting of transitions at third codon positions of the cytochrome *b* gene. The latter is intended to downweight the possibly misleading effect of transitions (Meyer, 1994), which accumulate at high frequencies at third positions (TS/TV = 8.9), since most of them represent synonymous substitutions. The two parsimony approaches resulted in minor topological differences, only involving the position of *G. assimilis* among the North American *Gryllus* (compare Figs. 2 and 3). The merits of weighted parsimony were recently discussed by Cunningham (1997), suggesting that the method performs well in recovering the correct tree. Additional support for the topology shown in Fig. 3 is provided by ML and ME analyses based on the GTR + Γ model that explicitly allows for among-site rate heterogeneity and different substitution ratios. We therefore assume this topology as the best working hypothesis for this set of taxa and use it in subsequent analyses. Further examination of additional *Gryllus* species should be used to test the proposed phylogenetic hypothesis.

Gryllus Phylogeny and Comparison with Earlier Phylogenies

The *Gryllus* phylogeny reported in the present study has resolved most issues that were unresolved in the earlier phylogeny of Harrison and Bogdanowicz (1995). Their phylogeny identified two groups of species that clustered together independent of the tree-building algorithm: (1) *G. fultoni, G. veletis,* and *G. cayensis,* and (2) *G. firmus, G. ovisopis,* and *G. pennsylvanicus.* However, the positions of two other North American species, *G. rubens* and *G. assimilis,* and the two European species, *G. bimaculatus* and *G. campestris,* differed considerably, depending upon which tree-building program was used. Moreover, the basal branching pattern was not well resolved among the *Gryllus* clades. Results of our phylogenetic analysis clearly separate

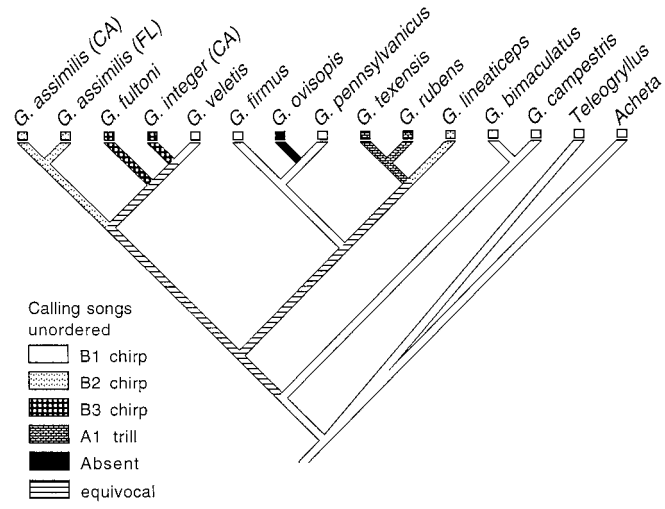

FIG. 5. Evolution of calling songs. The most-parsimonious reconstruction of calling-song characters is based on the tree presented in Fig. 3.

North American *Gryllus* species into four well-supported clades (numbered 1–4 in Fig. 3). Our best supported hypothesis (Fig. 3) suggests that the four clades form two groups, each containing two groups. Two of these clades (2 and 3, Fig. 3) correspond to the two clades that were consistently found by Harrison and Bogdanowicz (1995). In our study, only the position of one species, *G. assimilis,* differed in the trees constructed by unweighted parsimony and maximum likelihood (Figs. 2 and 3, see Results).

In both our phylogeny and that of Harrison and Bogdanowicz (1995), the two European species, *G. bimaculatus* and *G. campestris,* are sister taxa. However, in Harrison and Bogdanowicz's (1995) phylogeny, the European clade is embedded within the clade of North American species in trees constructed by maximum parsimony using equal weighting or by neighbor joining. This position of the European clade was unexpected and required Harrison and Bogdanowicz (1995) to discuss various scenarios that would allow genetic exchange between European and North American cricket species after the severing of the last land connection between Europe and North America. The phylogeny reported in the present study removes this problem since the two European species are found in a well-supported clade that is the sister group to all North American crickets (Figs. 2 and 3). Furthermore, the amount of sequence divergence between the North American and European *Gryllus* lineages (0.101) was significantly greater than the range of sequence divergences among North American and among European species (0.052–0.068) (see Results). These sequence divergence values are consistent with a basal drift-vicariant event in the early diversification of *Gryllus* associated with the opening of the North Atlantic during the late Creataceous or early Tertiary (Smith *et al.*, 1994).

Our finding that *G. veletis* and *G. pennsylvanicus* are not sister species, but rather are found in different clades (Figs. 2 and 3), supports the most important result of Harrison and Bogdanowicz (1995). Earlier cytological studies by Lim *et al.* (1973) and subsequent mtDNA sequence data obtained for *G. veletis, G. ovisopis, G. pennsylvanicus,* and *G. firmus* (Willet *et al.*, 1997) also support the notion that *G. veletis* and *G. pennsylvanicus* are not sister species. These data are inconsistent with Alexander's (1968) hypothesis that the different life cycles (nymphal vs egg diapause) found in *G. veletis* and *G. pennsylvanicus* evolved by allochronic speciation, since this hypothesis requires that *G. veletis* and *G. pennsylvanicus* be sister species.

Phylogenetic Relationships between Eastern and Western North American Gryllus

Western species of *Gryllus* have been much less studied than their Eastern North American counterparts. Harrison and Bogdanowicz (1995) showed that several unidentified western species, thought to be *Gryllus,* clustered with *G. veletis* in their phylogeny. Weissman *et al.* (1980) described nine western species, three of which were thought to be conspecific with species from the Eastern United States, but did not investigate their phylogenetic relationships. Our analysis indicates no phylogenetic correlate to the East-West distribution of North American *Gryllus* species. A single lineage (clade 1 in Fig. 3) contains only eastern species, while all others (clades 2–4) contain both eastern and western species. Individuals of *G. assimilis* from Florida and California appear to be members of the same species. The sequence divergence between these two taxa (0.006, estimated from the linearized tree) was lower than the minimum divergence between any pair of *Gryllus* species (0.008, between *G. pennsylvanicus* and *G. ovisopis*). Very little is known about the history of, or degree of current genetic interchange (if any) between, California and Florida *G. assimilis.* This species is widely distributed throughout the West Indies, Central American countries bordering on the Caribbean, and most of Mexico (Alexander and Walker, 1962; Weissman *et al.*, 1980). Alexander and Walker (1962) argued that this species is possibly a recent immigrant to southern Florida, while Weissman *et al.* (1980) proposed that *G. assimilis* has not been recently introduced into California.

Gryllus texensis was named while the present study was in progress (Cade and Otte, 2000). Crickets of this species were previously classified as *G. integer,* a species that had been described from individuals collected in California (Scudder, 1901, cited in Weissman *et al.*, 1980). It had long been suspected that *G. integer* from California and "*G. integer*" from Texas were different species. Calling song differed between "*G. integer*" from these two localities, and, in the laboratory, *G. integer* from California were unable to hybridize with "*G. integer*" from Texas (Weissman *et al.*, 1980; Smith and Cade, 1987; Cade and Tyshenko, 1990). Our phylogenetic analysis shows that *G. integer* and *G. texensis* occur in widely separated clades (Figs. 2 and 3), and hence are clearly different species.

Evolutionary Implications of the Gryllus Phylogeny

Life-cycle evolution in crickets has been the subject of numerous studies because of its central importance in the adaptation of species of this group to seasonality in temperate regions (Masaki and Walker, 1987). Three types of life cycle occur in North American *Gryllus:* direct development, diapause during the egg stage, and diapause during the nymphal stage. Except for *G. firmus,* each species exhibits only one of these three life cycles (Masaki and Walker, 1987). Northern populations of *G. firmus* are composed of obligate egg diapausers, while individuals from North Carolina or Florida can overwinter in the egg, nymph, or adult stage (Walker, 1980; Masaki and Walker, 1987). Results of

our phylogenetic analyses of life cycle evolution in *Gryllus* (Fig. 4) confirm the major findings of the previous study of Harrison and Bogdanowicz (1995). Egg diapause appears to have a unique evolutionary origin in North American and European field crickets, while nymphal diapause may have evolved independently as many as three times in this group.

The evolution of cricket song also has been extensively studied because of its importance in mate recognition and as a character in cricket taxonomy (Fulton, 1952; Alexander, 1962, 1968; Loher and Dambach, 1989). Our phylogenetic analysis of cricket song (Fig. 5) also supports conclusions of Harrison and Bogdanowicz (1995) concerning the evolution of this trait. The B1 chirp (according to the classification of Alexander, 1962) is found in both European species of *Gryllus,* as well as in both outgroups, *Acheta domesticus* and *Teleogryllus oceanicus.* This phylogenetic distribution is consistent with the argument of Harrison and Bogdanowicz (1995) that the B1 chirp is ancestral in *Gryllus.* The B3 chirp appears to have a unique evolutionary origin, while the B1 ancestral pattern has persisted through many speciation events.

CONCLUSIONS

The combined mtDNA data analyzed in this work provide a robust phylogenetic hypothesis for an important group of species of North American field crickets. This hypothesis, based on independent molecular information, is a timely contribution that allows unbiased interpretation of well-documented interspecific variation in behavioral and life-cycle traits. It will be interesting to see whether more comprehensive taxonomic sampling and additional genetic evidence (i.e., nuclear genes) will support the working hypothesis developed in this study. Regardless of the outcome, the phylogenetic basis to stimulate further comparative studies of *Gryllus* life history characters has been established.

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