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

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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 Gryllus integer Scudder 1901 from Texas to Gryllus 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...
TABLE 1

Gryllus Species and Outgroup Taxa Included in This Study

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection locality</th>
<th>GenBank Accession*</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. assimilis</td>
<td>Homestead, Florida</td>
<td>AF248657-8, AF248684</td>
</tr>
<tr>
<td>G. assimilis</td>
<td>Santa Barbara, California</td>
<td>AF248655-6, AF248683</td>
</tr>
<tr>
<td>G. bimaculatus</td>
<td>Cyprus</td>
<td>AF248659-60, AF248685</td>
</tr>
<tr>
<td>G. campestris</td>
<td>Germany</td>
<td>AF248661-2, AF248686</td>
</tr>
<tr>
<td>G. firmus</td>
<td>Gainesville, Florida</td>
<td>AF248663-4, AF248687</td>
</tr>
<tr>
<td>G. integer</td>
<td>Davis, California</td>
<td>AF248667-8, AF248689</td>
</tr>
<tr>
<td>G. texensis</td>
<td>Austin, Texas</td>
<td>AF248669-70, AF248690</td>
</tr>
<tr>
<td>G. fultonii</td>
<td>Gainesville, Florida</td>
<td>AF248665-6, AF248688</td>
</tr>
<tr>
<td>G. lineatipes</td>
<td>Santa Barbara, California</td>
<td>AF248671-2, AF248691</td>
</tr>
<tr>
<td>G. ovisopis</td>
<td>Gainesville, Florida</td>
<td>AF248673-4, AF248692</td>
</tr>
<tr>
<td>G. rubens</td>
<td>Gainesville, Florida</td>
<td>AF248676-7, AF248694</td>
</tr>
<tr>
<td>G. veltis</td>
<td>Sharon, Connecticut</td>
<td>AF248678-9, AF248695-6</td>
</tr>
<tr>
<td>G. pennsylvanicus</td>
<td>Ithaca, New York</td>
<td>AF248675, AF248693</td>
</tr>
<tr>
<td>Teleogryllus Oceanicus</td>
<td>Australia</td>
<td>AF248680-1, AF248697</td>
</tr>
<tr>
<td>Acheta domestica</td>
<td>Unknown*</td>
<td>AF248682, AF248698</td>
</tr>
</tbody>
</table>

* Cytochrome b and 16S fragment, respectively.

**Table 1** Gryllus species and outgroup taxa included in this study.

(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 MgCl₂, and 0.5 units of Taq polymerase (Gibco BRL). After checking PCR products on 1.5% agarose gels, they were purified using Wizard PCR Prepss (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

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’-3’)*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>P21</td>
<td>CCATCCAAACATCTCAGCATGATGAAA</td>
<td>Kocher et al. (1989)</td>
</tr>
<tr>
<td>P22</td>
<td>GCCCCCTCAGAATGATTTTGTCTCCTAC</td>
<td>Kocher et al. (1989)</td>
</tr>
<tr>
<td>2F</td>
<td>GTAATTAGCAACACGCTTATTAG</td>
<td>This study</td>
</tr>
<tr>
<td>2F</td>
<td>GTTATAGCTGACCGCTTTATTG</td>
<td>This study</td>
</tr>
<tr>
<td>2R</td>
<td>CCWARTTTTAGGAAATAGTCG</td>
<td>This study</td>
</tr>
<tr>
<td>3F</td>
<td>CAGCGWAYCCTTTATGAC</td>
<td>This study</td>
</tr>
<tr>
<td>3F</td>
<td>TAGGAGATCCAGATAATTTTAC</td>
<td>This study</td>
</tr>
<tr>
<td>3R</td>
<td>GCTTWKTYAAGCTMATTAACTTAA</td>
<td>This study</td>
</tr>
<tr>
<td>16Sb</td>
<td>CCCGTCCTGAACTCAGATCAG</td>
<td>Palumbi (1996)</td>
</tr>
</tbody>
</table>

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

### TABLE 3

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

^a Only one sequence per species was used.

^b Probability associated with the test for significant phylogenetic structure.
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 + I; 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 α = 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 + I 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-
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. 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.](image-url)
clades. The substitution model TrN + I' (Tamura and Nei, 1993) was used to estimate genetic divergence since it was the closest alternative in LINTRE to the GTR + I 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 $0.101 \pm 0.004$. Divergence among European species (height of node B in Fig. 3) was $0.052 \pm 0.010$, and the largest divergence among the North American species (height of node C in Fig. 3) was $0.068 \pm 0.002$. 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
the variation in cytochrome b was observed at third codon positions. Both data sets exhibit significant (non-random) 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 down-weight 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 + I 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. ovatusis, 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. campesiris, 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
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 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 divergence values are consistent with a basal drift species (0.052–0.068) (see Results). These sequence divergences among North American and among European 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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