Evidence for Cocladogenesis Between Diverse Dictyopteran Lineages and Their Intracellular Endosymbionts

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Bacteria of the genus *Blattabacterium* are intracellular symbionts that reside in specialized cells of cockroaches and the termite *Mastotermes darwiniensis*. They appear to be obligate mutualists, and are transmitted vertically in the eggs. Such characteristics are expected to lead to equivalent phylogenies for host and symbiont, and we tested this hypothesis using recently accumulated data on relationships among termites and cockroaches and their *Blattabacterium* spp. Host and symbiont topologies were found to be highly similar, and various tests indicated that they were not statistically different. A close relationship between endosymbionts from termites and members of the wood-feeding cockroach genus *Cryptocercus* was found, supporting the hypothesis that the former evolved from subsocial, wood-dwelling cockroaches. The majority of the *Blattabacterium* spp. sequences appear to have undergone similar rates of evolution since their divergence from a common ancestor, and an estimate of this rate was determined based on early Cretaceous host fossils. The results support the idea that the stem group of modern cockroaches radiated sometime between the late Jurassic and early Cretaceous—not the Carboniferous, as has been suggested on the basis of roach-like fossils from this epoch.

Introduction

Cockroaches, termites, and mantids form the well-accepted monophyletic group Dictyoptera (Marks and Lawson 1962; Kristensen 1981; Klass 1995; Lo et al. 2000). Intracellular *Blattabacterium* spp. are found in all cockroaches so far examined, as well as in the termite *Mastotermes darwiniensis* (Jucci 1952; Dasch, Weiss, and Chang 1984). They reside in specialized cells (mycetocytes or bacteriocytes) of the fat body, and are transferred vertically to the offspring via the eggs (Sacchi et al. 2000). Based on small subunit ribosomal DNA (16S rDNA) comparisons, they form a coherent cluster within the *Cytophaga-Flavobacterium-Bacteroides* (CFB) bacterial assemblage (Bandi et al. 1995), and cloning/sequencing analyses indicate that endosymbionts within a host are genetically uniform (Bandi et al. 1994).

Cockroaches that have had their endosymbionts removed via antibiotic treatment grow poorly, die easily, and lay fewer and less viable eggs (Guthrie and Tindall 1968). The interactions between host and symbiont have not yet been fully established, but they appear to include the recycling of uric acid by the bacteria (Cochran 1985; Wren and Cochran 1987), presumably in return for accommodation and metabolic products provided by the host. This suggests that the relationship is one of mutualism (Douglas 1989). Such a relationship is expected to lead to cocladogenesis of host and symbiont, though horizontal transfer events—which can be mediated by parasites—may lead to different phylogenetic topologies. Evidence has been found for phylogenetic congruence between *Blattabacterium* spp. and members of the genus *Cryptocercus* (Clark et al. 2001); however, a formal examination of whether cocladogenesis has occurred at deeper taxonomic levels of Dictyoptera has not yet been performed. Reasons for this have been (1) the absence of a suitable outgroup for rooting *Blattabacterium* spp. topologies; (2) the absence of a host phylogeny that included a variety of cockroaches, termites, and mantids; (3) the fact that *Blattabacterium* spp. from each of the five traditionally recognized lineages of cockroaches (McKittrick 1964) have not yet been examined.

The situation regarding suitable outgroups for *Blattabacterium* symbionts has improved with the discovery of CFB intracellular bacteria from ladybird beetles (Hurst et al. 1997). The 16S rDNA sequences of these endosymbionts share ~92% identity with those from various *Blattabacterium* spp., compared with ~80% in the previously known closest relatives (Bandi et al. 1995). With regard to dictyopteran phylogeny, two recent studies included members of each of the five cockroach families, as well as multiple termite and mantid representatives: that of Klass (1995), based on morphology, and that of Lo et al. (2000), based on molecular sequences. Both studies found evidence that mantids were the earliest branching lineage in the group, with termites sister to *Cryptocercus* spp. and nested within a paraphyletic cockroach grade. An alternative scenario is that cockroaches (including *Cryptocercus*) are monophyletic and a sister group to the mantids, with termites basal. This topology has found support from both morphological (Thorne and Carpenter 1992) and molecular (Kambhampati 1995) analyses. In addition, cockroach monophyly has been supported by a number of studies (Grandcolas 1996; Kambhampati 1996; Maekawa and Matsumoto 2000).

This recent accumulation of data provides an opportunity to examine whether the relationships among *Blattabacterium* spp. are congruent with those of their hosts. In this study, we performed the first outgroup-rooted, multifamily phylogeny of these bacteria and compared it to a phylogeny of host taxa based on a combined analysis of sequences from four genes. 16S rDNA sequences have been determined from *Blattabacterium* representatives of various cockroach families, including the Polyphagidae—the only cockroach family (sensu McKittrick 1964) that has not previously been examined.

Key words: symbiosis, molecular clock, cockroach, fossil, termite, *Blattabacterium*.

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Of particular interest with regard to dictyopteran phylogeny is the fossil record for these insects. Cockroach-like fossils are well known from late Carboniferous deposits (~300 Myr old). The key characteristic setting these fossils apart from extant cockroaches is the presence of a long, external ovipositor. Cockroach fossils which lack an ovipositor and that fit into extant families appear in early Cretaceous deposits (~130 Myr old). It is during this period when mantid and termite fossils also appear for the first time. Some authors have asserted that modern cockroaches and those from late Carboniferous deposits are monophyletic, which would mean that mantids and termites also existed during the Carboniferous epoch (Balderson 1991; Kukalova-Peck 1991; Jarzembowski 1994). An alternative hypothesis—more in line with the fossil record—is that the stem group of extant cockroaches, mantids, and termites was a roach-like insect with a reduced ovipositor (a characteristic of all dictyopterans) that radiated during the late Jurassic/early Cretaceous (Grimaldi 1997; Nalepa and Bandi 2000; Thorne, Grimaldi, and Krishna 2000). Divergence dates based on the rate of molecular evolution of endosymbiont genes (Moran et al. 1993) could provide evidence for or against the hypotheses mentioned above. We used data from cockroach and termite fossils to examine the rate of molecular evolution of the 16S rDNA gene in *Blattabacterium* spp., and we provide estimates of the splitting times between various lineages. Also, 16S rDNA rate evolution from endosymbionts of the CFB bacterial assemblage is of interest because it provides a comparison to previously determined fossil-based rate estimates, which, to our knowledge, have all been from the proteobacterial assemblage (Ochman, Elwyn, and Moran 1999).

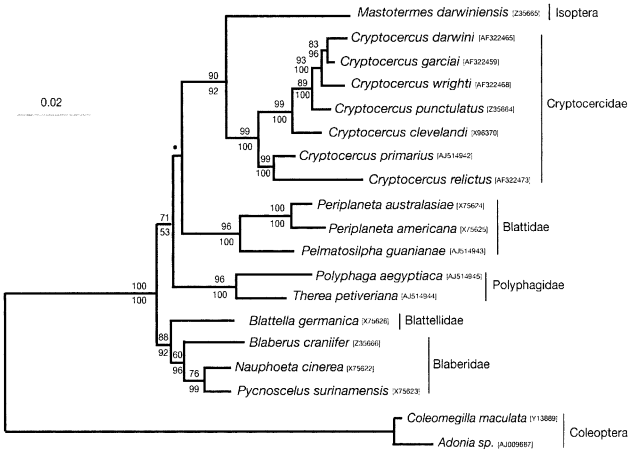

FIG. 1.—Phylogeny of *Blattabacterium* spp. from representatives of each of the five cockroach families as well as the termite *Mastotermes darwiniensis*, based on 16S rDNA sequences. Species names are those of the hosts. Note that current evidence for species-level status for *C. darwini*, *C. garciai*, and *C. wrighti* may be premature (Nalepa et al. 2002). Topology shown is the maximum parsimony (MP) tree obtained via a heuristic search. Tree length: 549, consistency index: 0.68, branch lengths fitted by ML analysis. Values above and below branches are bootstrap and posterior probability percentages from MP and Bayesian analyses, respectively. The bullet indicates a node that had less than 50% support from both analyses. Trees were rooted with 16S rDNA sequences from intracellular bacteria from two ladybird beetle species. Accession numbers for each bacterial sequence are shown adjacent to host species names. Scale bar indicates inferred substitutions/site.

Materials and Methods

Samples of the visceral fat body were obtained from wild-collected cockroach specimens of *Polyphaga aegyptiaca* (Polyphagidae), *Therea petiveriana* (Polyphagidae), *Cryptocercus primarius* (Cryptocercidae), and *Pelmatosilpha guanianae* (Blattidae). Crude DNA preparation, polymerase chain reaction (PCR) with universal primers, and direct sequencing of ~1400 bp of the amplified bacterial 16S rDNAs were performed as previously described (Bandi et al. 1994). Sequences were deposited into the European Molecular Biology Laboratory (EMBL) database; accession numbers are shown in figure 1. Manual alignment with previously reported 16S rDNAs of *Blattabacterium* spp. and intracellular bacteria of the ladybird beetles *Coleomegilla maculata* and *Adonia* sp. (outgroups) was performed, using pre-aligned sequences (Neefs et al. 1993) as a guide. For sequences from some *Cryptocercus* spp., a region of only ~1200 bp was available (Clark et al. 2001), and thus the remaining part of the sequence was treated as missing in phylogenetic analyses (except those involving clock-tests, where this last region was excluded; see below).

Phylogenetic analyses were performed under maximum parsimony (MP), maximum likelihood (ML), and Bayesian criteria. To verify that the data set contained significantly more phylogenetic structure than random data, we measured the skew (g_1) in the distributions of tree lengths, based on 1,000 randomly generated trees (''generate trees'' option in PAUP*4.0b10 [Swofford 2000]). The significance of g_1-values was assessed using the critical values for four-state character data listed previously (Hillis and Huelsenbeck 1992). For likelihood analysis, the most appropriate model of sequence evolution was determined via likelihood ratio tests (5% significance level) using the program Modeltest 3.06 (Posada and Crandall 1998). To check for potential variations in base composition among the sequences in each dataset, the chi-squared test for stationarity in Tree-Puzzle 5.0 was used (Strimmer and von Haeseler 1996).

Tree topologies were estimated by MP using heuristic searches in PAUP*, treating gaps as a fifth base. Branch lengths were fitted using ML criteria. Support for internal nodes was estimated by bootstrap analysis (1,000 replicates, with 5 random addition replicates per bootstrap replicate) and by Bayesian inference using the program MrBAYES 2.01 (Huelsenbeck and Ronquist 2001). For Bayesian analyses, parameters for the selected model of substitution were estimated from the data, and a total of 12,000 trees were obtained (ngen = 12, 0000, samplefreq = 10). The first 6,000 of these were considered as the ''burn in'' and discarded, and a 50% majority-rule consensus tree of the remaining trees was produced. Clock-like evolution in the 16S rDNA of the taxa examined was tested for using the option in Tree-Puzzle 5.0.

To test for congruence, a phylogeny for the hosts was estimated based on four genes that have been sequenced for various taxa (nuclear 18S rDNA, mitochondrial 12S rDNA, 16S rDNA, and cytochrome oxidase II; see

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Supplementary Material). The rDNA sequences were aligned based on secondary structure considerations as outlined previously (Kambhampati 1996; Lo et al. 2000; Thompson et al. 2000). Regions that could not be aligned unambiguously were discarded from analyses. To check that the four genes were suitable to be combined with one another, 50% majority-rule consensus trees were obtained via MP bootstrap analysis for each individual data set. Upon comparison of these trees, no conflicting clades were found, and thus combined analyses were performed using the methods described above for endosymbiont analyses. Taxa included were those which had at least three of the four genes available in GenBank. For two genera (*Blattella* and *Blaberus*), genes from closely related species within the one genus were combined. Four mantid species, for which only two genes were available, were also included. Two Orthopteran taxa were used as outgroups. Data unavailable for genes of some taxa were coded as missing.

Three statistical tests of phylogenetic congruence between *Blattabacterium* spp. and their hosts were performed. The first tested whether there was a greater than random correspondence between reconstructed nodes for host and symbiont. This was performed in Component Lite (R. Page, University of Glasgow, UK) using the ''compare tree with'' function, with 1,000 randomized trees and the four available tree-comparison metrics. The second test examined the null hypothesis that the endosymbionts have undergone cocladogenesis with their hosts. The most-parsimonious tree and its length were first calculated for each of the host and endosymbiont data sets. The MP tree of one data set was then forced onto the other data set, and the tree-lengths were calculated. The two tree-lengths calculated for a single data set were then compared using the Templeton (1983) test. Significantly different scores can be interpreted as rejection of the null hypothesis. The third test was similar to the second test, except that ML criteria were used. ML trees were estimated using the successive approximation method (Swofford et al. 1996), and the different scores for the one data set were compared using the Shimodaira and Hasegawa (1999) test. This test is similar to that used by Peek et al. (1998) and Clark et al. (2000).

Results and Discussion

Phylogenetic Relationships Among *Blattabacterium* spp.

All 16S rDNA sequences obtained were unambiguous, indicating that a single bacterial type was present within a host. Owing to the presence of few insertions/deletions, alignment of the sequences with previously determined *Blattabacterium* spp. sequences was straightforward (see *Supplementary Material*). Significant phylogenetic signal was found, with a skew value ($g_1 = -1.55$) below the critical value for significance at the $P < 0.01$ level (Hillis and Huelsenbeck 1992). Of 1,409 characters examined, 303 were found to be variable and 215 parsimony informative. None of the sequences were found to differ significantly in base composition. The average base compositions for all *Blattabacterium* spp. were as follows A: 28.99 ± 0.09, C: 18.93 ± 0.08, G: 27.84 ± 0.06, T: 24.24 ± 0.06 (mean $\pm$ SD; $n = 17$). Thus the base composition of this gene was not strongly skewed toward AT (53.23%). The most appropriate model of nucleotide substitution for likelihood analyses selected by Modeltest 3.06 was ''HKY + I + G.''

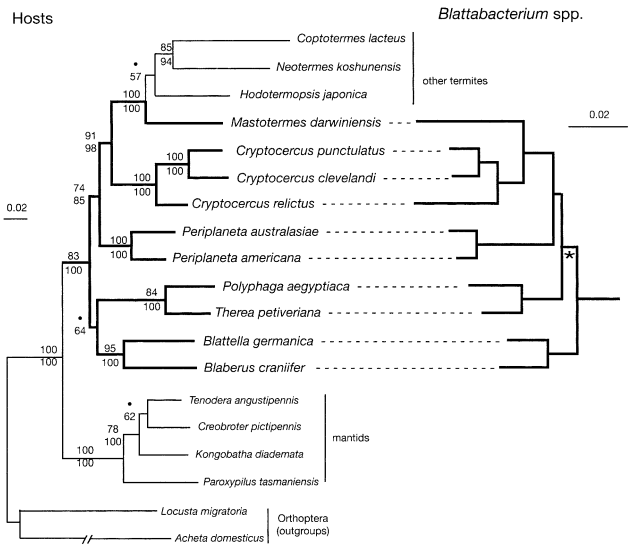

FIG. 2.—Phylogeny of dictyopteran species and a comparison with the phylogeny of *Blattabacterium* spp. The host phylogeny was based on a combined analysis of 18S rDNA and mitochondrial COII, 12S rDNA, and 16S rDNA sequences. The topology shown is the MP tree obtained via a heuristic search. Tree length: 2901, consistency index: 0.55. Support values and other details are as given in figure 1. Bold lines indicate those dictyopteran taxa that harbor *Blattabacterium* spp., and that were examined in host–endosymbiont congruence tests. The phylogeny of *Blattabacterium* spp. was based on those species for which the host phylogeny was performed, to allow statistical testing of congruence. Support values and other characteristics of this tree were very similar to those in figure 1. The asterisk indicates the only node in the topology that was in disagreement with that based on the host phylogeny. Outgroups were as for figure 1 but are not shown.

Figure 1 shows the most-parsimonious tree recovered from 16S rDNA analysis. The 50% majority-rule consensus tree obtained from Bayesian analysis was identical to figure 1, with the exception that one node was not resolved. Consistent support was found for the monophyly of *Blattabacterium* spp. from each of the cockroach families Cryptocercidae, Blattidae, Polyphagidae, and Blaberidae (for Blattellidae, only one sequence was available). A clade containing endosymbionts from the termite *M. darwiniensis* and from *Cryptocercus* spp. was well supported in both bootstrap (90% of replicates) and Bayesian (92% posterior probability) analyses. Sequences from Blaberidae and Blattellidae were found to cluster together, in agreement with results from several studies of cockroach relationships (McKittrick 1964; Klass 1995; Grandcolas 1996; Kambhampati 1996; Maekawa and Matsumoto 2000). The exclusion of the few gap positions present in the alignment did not alter the topologies recovered from MP or Bayesian analysis, and similar support values were found (data not shown).

Examining Congruence Between Symbiont and Host Topologies

Figure 2 shows a comparison of the phylogeny of a subset of endosymbionts with that of their hosts (for

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Table 1
Testing the Null Hypothesis of Cocladogenesis Between Hosts and Endosymbionts

	Templeton Test (parsimony based; compares tree lengths)			Shimodaira-Hasegawa Test (likelihood based; compares $-\ln L$ scores)		
Topology:	*Blattabacterium* (fig. 2)	Host (fig. 2)	Grandcolas 1996[a]	*Blattabacterium* (fig. 2)	Host (fig. 2)	Grandcolas 1996[a]
Score based on *Blattabacterium* data set	457	461	480	−4103.84	−4104.67	−4141.04
P value	Best	$P = 0.16^{ns}$	$P < 0.01$*	Best	$P = 0.69^{ns}$	$P < 0.01$*
Score based on host data set	2017	2016	2045	−12615.19	−12614.19	12672.25
P value	$P = 0.85^{ns}$	Best	$P < 0.01$*	$P = 0.99^{ns}$	Best	$P < 0.01$*

NOTE.—The superscript ns indicates not significantly different from the shortest tree length (parsimony) or highest likelihood score for a given data set; an asterisk indicates significantly longer tree length (parsimony), or significantly lower likelihood score.

[a] (O, (Md, (Peri, ((Bg,Bc), (Pa, (Cryp,Tp)))))), where O = outgroup; Md = *M. darwiniensis*; Peri = *Periplaneta* spp.; Bg = *B. germanica*; Bc = *B. craniifer*; Pa = *P. aegyptiaca*; Cryp = *Cryptocercus* spp.; Tp = *Therea petiveriana*.

which molecular data are available). From the four host genes, 2,991 characters were analyzed, of which 1,067 were variable and 674 were parsimony informative. Significant phylogenetic structure was found for each of the individual data sets (data not shown), as well as the combined data set ($g_1 = -0.76$; $P < 0.01$). For ML analyses, the model of substitution chosen by Modeltest was GTR + G. We note that PAUP* does not permit the application of different models for individual data sets within a combined data set. Attempts with a program that allows for this (PAML; Yang 1997) proved computationally intractable.

In addition to congruence at the within-family level, congruence is found at deeper levels of the topologies (for example, the termite/*Cryptocercus* spp. grouping). Analyses in Component Lite showed that there was a significant level of similarity between the host and bacterial topologies, with a very low probability of obtaining the observed number of shared nodes by chance alone ($P < 0.001$ for all tree comparison metrics). The grouping of Polyphagidae with Blattellidae + Blaberidae was found in the host phylogeny but not in the endosymbiont phylogeny, where Polyphagidae was placed more apically. Is this conflict statistically significant, or can it be explained by stochastic error in the data? The results of statistical comparisons of parsimony tree-lengths and likelihood scores—when the best topology of the host data set was forced onto the endosymbiont data set and vice versa—are shown in table 1. In all comparisons, the differences in tree lengths and likelihoods were not found to be statistically significant. No evidence was found for any among-family horizontal transfer or host-switching between any of the taxa examined, and we were therefore unable to reject the null hypothesis that cocladogenesis has occurred throughout the evolutionary history of hosts and their symbionts.

The morphology-based topology of Grandcolas (1994, 1996)—which has *Cryptocercus* species as derived members of the Polyphagidae—was also forced onto both host and endosymbiont data sets for comparative purposes (see table 1). The resulting tree-lengths were significantly longer than those for the best topologies for each data set based on the Templeton test. Similarly, the likelihood scores were significantly lower than the ML scores for each data set based on the Shimodaira-Hasegawa test. The results of the analyses on the host data set call into question the phylogenetic scenarios proposed by Grandcolas, and are in agreement with a recent study by Klass (2001), which refuted almost all the autopomorphies used by Grandcolas to infer his phylogeny.

Testing for Clock-Like Behavior in *Blattabacterium* spp. 16S rDNA, and Estimating Its Rate of Evolution

The evidence for cocladogenesis and the presence of fossil data for termites and cockroaches prompted us to investigate the rate of evolution in the 16S rDNAs of *Blattabacterium* spp. If the sequences evolve in a roughly constant manner, the rate determined for a subset of sequences using fossil data can be used to date the divergences of other taxa. Clock-like evolution in the sequences used to generate figure 1 was tested for using the likelihood-ratio–based test available in Tree-Puzzle 5.0. The null hypothesis that the sequences have evolved in a clock-like manner was rejected at the 5% level (data not shown). However, it was apparent from the branch lengths in figure 1 that such rejection may have been caused by a minority of lineages that have undergone changes in rate. Indeed, when the lineages leading to Blaberidae/Blattellidae and *C. relictus* were removed from the analysis (5 of 17 taxa), the null hypothesis of clock-like evolution could not be rejected. The log likelihood score of the tree where branch lengths were free to vary was –3906.11, whereas that constraining them to be clock-like was –3914.22. These values were not significantly different at the 5% level ($P = 0.133$). We note that when taxa other than Blaberidae/Blattellidae and *C. relictus* were removed singly or in combination, the null hypothesis of clock-like evolution was consistently rejected. Thus we conclude that these two lineages are responsible for all significant among-lineage rate heterogeneity.

Because the *Mastotermes*/*Cryptocercus* spp. clade is supported by morphological (Klass 1995) and molecular data from the hosts (fig. 2; Lo et al. 2000), as well as endosymbiont molecular data (fig. 1), it is a good candidate for calibration of the 16S rDNA clock. Though no *Cryptocercus* fossils are known, the earliest known fossils for termites are from the early Cretaceous (Thorne, Grimaldi, and Krishna 2000). We can therefore assume that the latest possible time of splitting between the

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lineages leading to *Cryptocercus* spp. and *M. darwiniensis* was ~130 MYA. The average ML distance between the 16S rDNAs from *M. darwiniensis* and *Cryptocercus* species (excluding *C. relictus*) is 4.5% ± 0.4% (mean ± SD; $n = 6$). Thus, with the assumption of a molecular clock, 0.0225 substitutions per site have occurred in each of these lineages since their last common ancestor. Based on a minimum divergence date of 130 myr, we can estimate that the maximum rate of evolution is 0.0087 substitutions per site per 50 myr. This rate is within the range of 0.0076 to 0.0232 substitutions per site per 50 myr previously reported for the 16S rDNA sequences of aphid symbionts (Moran et al. 1993), despite being from a phylogenetically diverse bacterial group. Using the rate calculated in our study, the latest time of divergence between the termite/*Cryptocercus* lineage and Blattidae (for which endosymbiont 16S rDNA ML distances average 5.0% ± 0.5% [$n = 21$]) is 144 MYA. The appearance of Blattidae fossils in the early Cretaceous is in agreement with this value (Labandeira 1994). Similar results are found when examining distances between various taxa and Polyphagidae members, for which early Cretaceous fossils are also known (Labandeira 1994).

The branch lengths in figure 1 suggest that 16S rDNAs from the lineage comprising Blattellidae/Blaberidae have evolved in a slower manner compared with those from the other lineages. Because of these different rates, it is difficult to estimate the period when the last common ancestor to all families existed. Based on the short branch separating Blaberidae/Blattellidae from the other taxa (fig. 1), it could be speculated that this stem group was present at a time similar (but slightly earlier) to the value of ~140–145 MYA calculated for the split between termites/*Cryptocercus*, Blattidae, and Polyphagidae based on ML distances. Other genes that have evolved in a clock-like manner for all taxa will need to be examined to test this idea.

The above evolutionary rate calculation and its corroboration from other fossil taxa relies on the assumption that the fossil record adequately reflects the first appearance of the lineages examined. In support of this assumption is the combination of three observations: (1) The oldest termite, Polyphagidae, Blattidae, and Blattellidae fossils are all from the early Cretaceous. (2) Roach-like fossils are found from the Carboniferous up to the late Jurassic, and contain ovipositors that gradually decrease in length over time (Thorne, Grimaldi, and Krishna 2000). If ovipositorless roaches resembling modern taxa also existed during these periods, it is difficult to explain why their fossils are not found as well. (3) The branch lengths that separate the main modern lineages are short in both the endosymbiont and host phylogenetic trees (indicative of a relatively rapid radiation from a common ancestor).

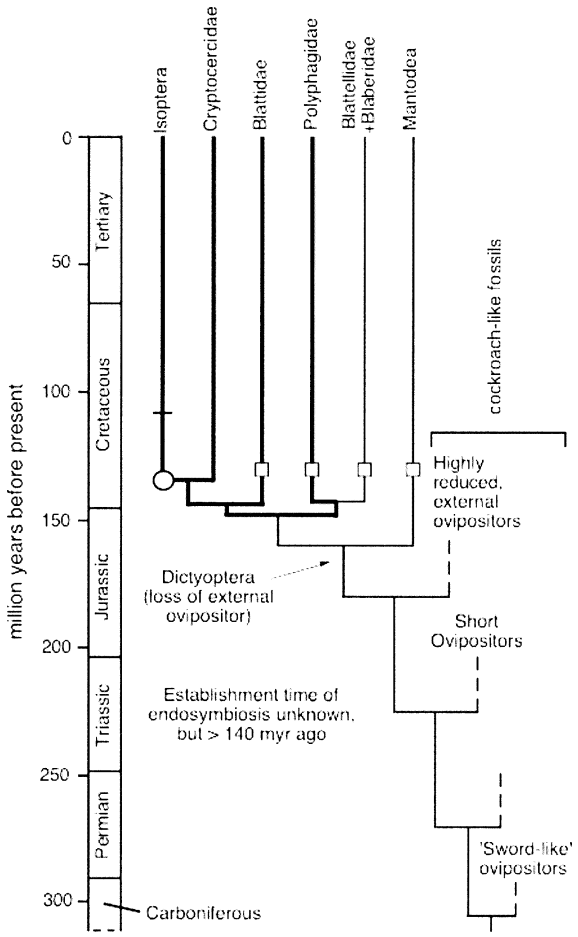

FIG. 3.—Timescale for the evolution of Dictyoptera. Ovipositor length of cockroach-like fossils decreases in time from Carboniferous specimens through to those in the late Jurassic, where they become highly reduced. The relationships shown among these fossils are taken from Grimaldi (1997). The relationships within Dictyoptera are based on those in figure 2. Clock-like behavior in the 16S rDNA of *Blattabacterium* spp. from lineages in bold permitted their approximate times of divergence to be determined (see text). Calibration for the rate of *Blattabacterium* 16S rDNA evolution was based on early Cretaceous termite fossils, indicated by a circle. Squares indicate the presence of the earliest known fossils within other lineages, also from the early Cretaceous. The 16S rDNA in the Blaberidae + Blattellidae lineage has apparently evolved in a slower manner than those in bold, and thus its time of divergence from the latter is not clear. The lack of endosymbionts in the Mantodea lineage precludes an estimation of its divergence time, and the time shown is based on Grimaldi (1997). The bar on the termite lineage represents loss of *Blattabacterium* from all other lineages other than that leading to *M. darwiniensis* (see also fig. 2).

Conclusions

A summary of the results of this study is given in figure 3. Cockroach, termite, and mantid fossil data all suggest that the Dictyoptera evolved from roach-like insects with reduced ovipositors sometime during the late Jurassic and early Cretaceous (Grimaldi 1997), and divergence date estimates based on *Blattabacterium* 16S rDNA distances agree with this notion. Morphological (Klass 1995) and molecular data (Lo et al. 2000; this study) indicate that the first major split was between mantids and cockroaches, although further data are required to confirm this. The major cockroach lineages appear to have diverged within a relatively short period, based on endosymbiont and host molecular data. There is now good evidence that one lineage of wood-feeding cockroaches gave rise to termites and *Cryptocercus* spp.; however, relationships between the other major lineages

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are not yet resolved. It is not clear when the endosymbiosis was first established, but it appears to have been at least 140 MYA, based on our inability to reject the null hypothesis of cocladogenesis. It is conceivable that roach-like insects harbored *Blattabacterium* much earlier, and that mantids lost them when they acquired the predatory habit from their detritivorous ancestors, though this possibility is difficult to test. The loss of the bacteria from all other lineages of termites except that leading to *M. darwiniensis* (see fig. 2) may have been made possible by the development of a complex microbiota in the guts of the ancestors of these termites, which was able to assume the role that *Blattabacterium* presumably played in their metabolism. Indeed, bacteria in the guts of extant termites are known to be able to convert the nitrogenous end product uric acid into metabolites usable by the host (Breznak 2000).

Supplementary Material

A table showing GenBank Accession Numbers for the host sequences used in this study and an alignment of *Blattabacterium* sequences are available at the Web site for this journal.

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