Directional Substitution and Evolution of Nucleotide Content in the *Cytochrome Oxidase* II Gene in Earwigs (Dermapteran Insects)

*Thierry Wirth,**† *René Le Guellec,*‡ *and Michel Veuille**

*Laboratoire d'Ecologie and Ecole Pratique des Hautes Etudes, Paris 6 University, Paris, France; †Department of Integrative Biology, Section of Conservation Biology, University of Basel, Basel, Switzerland; and ‡Laboratoire de Biologie et Génétique du Développement, Centre National de la Recherche Scientifique Campus de Beaulieu, Rennes, France

The *cytochrome oxidase subunit* II (*CO*II) gene was sequenced for six dermapteran species. The nucleotide composition of this gene is biased in most animals. While the CG content of other insect orders is low (mean, 27.6%; range, 19.5%–33.1%), species from the *Forficula* genus showed unusually high values (mean, 42.4%; range, 37.3%–44.1%), mostly due to high CG frequencies at third codon positions: the mean CG content at these positions was around 45% (range, 43.9%–46.9%) for *Forficula,* compared with only 13.3% for other insects. This effect was so strong that in one species, *Forficula lesnei,* there was no significant difference between the frequencies of the four bases. During evolution, this loss of bias has involved a significant increase in the synonymous substitution rate and an increase of transitions over transversions compared with other insects. A strong directionality of substitutions has favored T→C and A→G changes. This phenomenon was also observed between two conspecific populations of *Forficula auricularia.* A species from a closely related genus, *Anechura bipunctata,* was intermediate between *Forficula* and other insects for these parameters, while two remotely related dermapteran species, *Labidura riparia* and *Euborellia moesta,* were similar to other insects. These results suggest that the evolution of *Forficula* DNA content has been both rapid and recent.

Introduction

The nucleotide content of DNA is a simple character. However, its evolution is probably controlled by a number of forces. Biases in mutational processes and repair mechanisms were first hypothesized by Sueoka (1962). Their role is apparent in compositional asymmetries between the two DNA strands, which may be produced by mutations during replication or transcription (reviewed by Francino and Ochman 1997). Selection can also be involved in mtDNA evolution. While adaptive mechanisms at the DNA level are suggested by the dependence of mitochondrial evolutionary rates on thermal habits (Rand 1994), attempts to explain biased DNA contents by the greater stability of the C:G bond at high temperatures are not supported by comparative data from bacteria (Galtier and Lobry 1997). Nucleotide composition appears to be a highly variable character. The CG content at third codon positions of the same gene can vary widely between related species, as, for example, in the many species of *Drosophila* (Moriyama and Gojobori 1992). Similarly, different genes from a given species can vary widely in codon usage (Sharp and Li 1989). The evolution of DNA composition also affects the amino acid content of gene products (Jermiin and Crozier 1994; Foster, Jermiin, and Hickey 1997). For a given set of amino acids, differences in codon usage are linked to gene expressivity (Grantham et al. 1981; Shields et al. 1988; Moriyama and Hartl 1993). Nucleotide content thus appears to be determined by an equilibrium between a great many forces, and one would not expect it to change dramatically and directionally in a single taxon. Yet, we report such a finding in this paper.

In the course of a study of mtDNA variation in the European earwig *Forficula auricularia* (Wirth et al. 1998), we sequenced a 627-bp mtDNA fragment partly overlapping the *cytochrome oxidase subunit* I (*CO*I) and *cytochrome oxidase subunit* II (*CO*II) genes and found significant differences in base composition between two sibling species. We also found an unusually high CG content which substantially extends the known range of variation of insect mitochondrial genes. In order to investigate this result, we sequenced the whole *CO*II gene for several dermapteran species. Results reported below show that a dramatic change in base composition has occurred in a dermapteran family, Forficulidae.

Key words: mtDNA, cytochrome oxidase II, codon usage, molecular phylogeny, Dermaptera, insects.

Address for correspondence and reprints: Michel Veuille, Ecole Pratique des Hautes Etudes and Centre National de la Recherche Scientifique, Laboratoire d'Ecologie, Case 237, Université Paris 6, 7 quai Saint-Bernard, 75252 Paris cedex 05, France. E-mail: mveuille@snv.jussieu.fr.

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Materials and Methods

Species Sample

Our sample consisted of six dermapteran species, four of them belonging to the Forficulidae (*Forficula auricularia, Forficula lesnei, Forficula decipiens,* and *Anechura bipunctata*), one belonging to the Labiduridae (*Labidura riparia*), and one belonging to the Carcinophoridae (*Euborellia moesta*). The labidurid and carcinophorid families have morphological traits that are considered primitive, while the Forficulidae are considered to be a monophyletic group with derived morphological traits. As will be shown below, molecular phylogenetic analysis confirms these views. Two populations of *F. auricularia* were studied: ''Mijanes-1500'' was collected in the Pyrenees, and ''Rome'' was collected in Italy. These two populations, although genetically and ecologically differentiated, belong to the same species (Wirth et al. 1998). We used them in order to detect recent changes in nucleotide content.

Table 1
Amplifying and Sequencing Primers Used in this Study

Name	Sequence	Positions[a]
EB	5′-GGGGATCCATACCACGACGTTATTCAGA-3′	2773–2792
ATPase	5′-CCGAATTCTCATCTTATAGGTACTATTTGAGG-3′	3914–3937
A-tLeu	5′-ATGGCAGATTAGTGCAATGG-3′	3018–3037
B-tLys	5′-GTTTAAGAGACCAGTACTTG-3′	3785–3804
HR	5′-CCGAATTCAATATCATTGATGACC-3′	3383–3398
Bi-med	5′-GGTACAGCTCAAGAATG-3′	3578–3594
Les-med	5′-ACCCCCAAAGCTGGGACTGCTCA-3′	3569–3591

[a] Positions are given according to the *Drosophila yakuba* sequence (Clary and Wolstenholme 1985).

DNA Preparation, Amplification, and Sequencing

Unless stated otherwise, all DNA handling was performed according to standard protocols (Sambrook, Fritsch, and Maniatis 1989). DNA was extracted as previously described (Wirth et al. 1998). PCR primers were designed in conserved regions (table 1) from an alignment of mitochondrial DNA sequences from *Drosophila yakuba* (Clary and Wolstenholme 1985), *Apis mellifera* (Crozier, Crozier, and McKinley 1989), and *Locusta migratoria* (Flook, Rowell, and Gellissen 1995). The amplified DNA fragment overlaps the *CO*I gene, the Leu-tRNA, the whole *CO*II gene, the Lys-tRNA, the Asp-tRNA, and the 5′ end of the *ATPase*-8 gene. It covers 1,165 bp corresponding to positions 2773–3937 of the *Drosophila melanogaster* mitochondrial genome (De Bruijn 1983). Following DNA amplification (Saiki et al. 1989), amplified products were purified in agarose, extracted using the Jet-Sorb extraction protocol (Genomed Inc.), and cloned into a Bluescript plasmid. DNA sequencing was performed in at least two clones per matriline using the dideoxy chain termination method (Sanger, Nicklen, and Coulson 1977). A set of internal sequencing primers (table 1) was used. Sequences were aligned using the ESEE sequence editor (Cabot and Beckenbach 1989). They are available from the GenBank and EMBL databases under accession numbers AF140539–AF140545.

Phylogenetic Analysis

The COII amino acid sequence was deduced from the DNA sequence using the *Drosophila* mitochondrial genetic code (Clary and Wolstenholme 1985). Nucleotide sequences were analyzed using version 1.01 of MEGA (Kumar, Tamura, and Nei 1993). The matrix of corrected DNA distances was generated using Kimura's (1980) two-parameter model, and a phylogenetic tree was produced using the neighbor-joining algorithm (Saitou and Nei 1987). Bipartitions in the neighbor-joining tree were examined by bootstrap analysis over 1,000 replicates (Felsenstein 1985).

Results

Overall Base Composition

The sequence alignment is given in appendix 1. The *CO*II gene consisted of 684 nt (228 codons) in all species except *E. moesta*, which has a deletion of one codon at position 679. The dermapteran *CO*II genes have substantial differences in their CG contents. The two species from nonforficulid families, *E. moesta* and *L. riparia*, have low CG contents (29.7% and 31.2%, respectively) which are within the range of values observed by Liu and Beckenbach (1992) for other insects (mean, 27.6%; range, 19.5%–33.1%). In the Forficulidae (*Forficula* and *Anechura*), the CG content was 37.3% in *A. bipunctata* and between 43.4% and 47.1% in *Forficula*. These values are the highest recorded for insects.

Base Composition at Each Codon Position

Figure 1 compares the CG contents of the Dermaptera and other insects at each codon position. It shows that the variation in nucleotide content almost completely resides in the third codon position. The forficulids appear to be very different from all other insects, including other Dermaptera.

Table 2 gives a detailed account of base composition at each of the three codon positions in the Dermaptera compared with the average content given by Liu and Beckenbach (1992) for other insects. No significant difference was observed at any position between nonforficulid Dermaptera and other insects. In the Forficulidae, the difference was significant ($P < 0.05$) at first codon positions in all species, and highly significant in *F. auricularia* ($P < 0.01$, df = 1, $\chi^2 = 5.17$ using Yates' correction). The difference was never found to be significant at the second position. It was highly significant ($P < 0.01$) in all species at the third position.

Index of Nucleotide Bias at Each Codon Position

Table 3 shows the bias in nucleotide content using Irwin, Kocher, and Wilson's (1991) index, which measures deviation from equal nucleotide frequency (25%) in A, C, G, and T. This index varies from 0% (when there is no bias) to 100% (for complete bias, when only one nucleotide is present). Nondermapteran insects showed small biases in first and second positions, and a strong bias in third positions (17.47-23.20-48.93 at the three positions successively). The same was found for nonforficulid Dermaptera *E. moesta* (12.20-22.00-48.40) and *L. riparia* (9.27-19.33-50.27). Forficulidae showed a dramatic decrease of bias in third positions, with the most extreme value being that for *F. lesnei* (10.00-17.20-7.60). In this species, the compositional bias at third positions did not significantly differ from the 25% frequency for all four nucleotides ($\chi^2 = 8.19$, df = 3, NS). The same test for other positions or species was always significant.

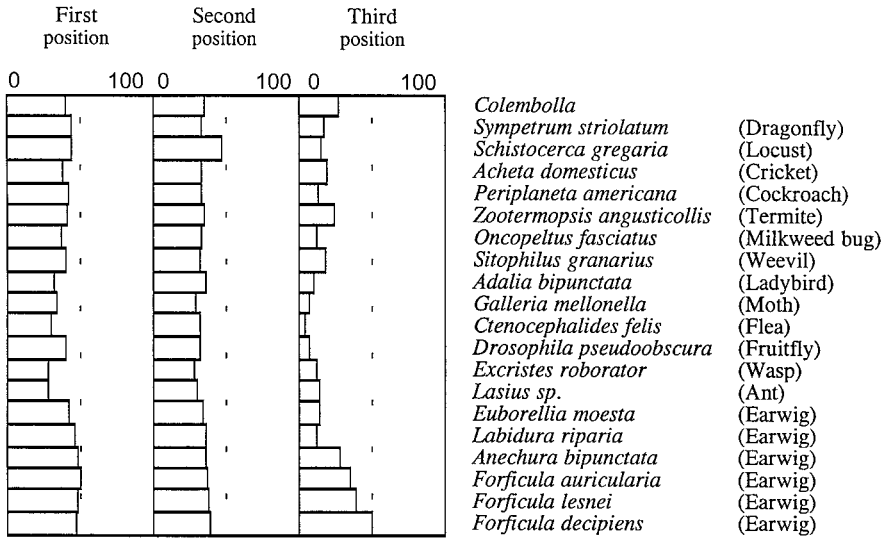

FIG. 1.—Percentages of CG content of first, second, and third codon positions in *CO*II for Dermaptera, compared with values observed in several insect species by Liu and Beckenbach (1992) and Frati et al. (1997).

Compositional Changes in A, C, G, and T Nucleotides

The decrease in the bias index does not necessarily imply that all four nucleotides tend to be at an equal frequency. This process was mostly accomplished through a switch in the balance of the two purines, A and G. The frequency of A over all positions decreased from 36.2% in other insect orders to about 25% (range, 23.1%–25.7%) in Forficulidae, whereas the frequency of G increased from 11.6% to about 25% (range, 22.5%–26.3%). In contrast, no systematic trend was observed in the variation of C (from 16.0% in other insects to 14.8%–22.3% in Forficulidae) and T (from 36.2% in insects to 31.2%–37.0% in Forficulidae). Nonforficulid Dermaptera were also different from other insects with regard to third positions, but they were completely different from the Forficulidae. They showed a lower proportion of C-terminating codons (4.0%–4.4% vs. 10.9% in other insects) and a higher proportion of T-terminating codons (50.2%–50.4% vs. 42.1% in other insects). We therefore observe that A decreases and G increases in the Forficulidae, while T increases and C decreases in other Dermaptera. Nonforficulid Dermaptera are probably representative of the dermapteran ancestral state (see the phylogenetic analysis below). Therefore, for the primitive Dermaptera, it is likely that both C and G increased in the Forficulidae, while A and T decreased.

Relative Importance of Transitions and Transversions

A classic observation is that transitions outnumber transversions between closely related species but not between distant ones (Brown and Simpson 1982). In this study, the two species from morphologically ''primitive'' families (*E. moesta* and *L. riparia*) were used as representatives of the ancestral condition among earwigs. Their low CG contents and their phylogenetic branching at the root of Dermaptera (see below) validate this approach. The directionality of nucleotide substitutions was characterized by comparing *E. moesta* with either *L. riparia* or *F. lesnei.* If evolutionary rates were the same, *E. moesta* would be equally different from the other two species. Table 4 shows the number of substitutions, including the relative number of transitions and transversions, between *E. moesta* and the other two species. The number of substitutions in first and second positions was the same in the two pairs of species. For the third position, there was a highly significant excess of substitutions in the pair involving *F. lesnei* ($\chi^2 = 9.60$

Table 2
Nucleotide Content (%) at First, Second, and Third Codon Positions of *CO*II

Species	First				Second				Third				Total
	A	C	G	T	A	C	G	T	A	C	G	T	C+G
Insects[a]	36.1	17.2	19.7	27.0	28.0	20.0	12.6	39.4	44.6	10.9	2.4	42.1	27.6
Euborellia moesta	28.2	15.9	26.0	30.0	26.9	18.1	15.4	39.6	36.1	4.0	9.7	50.2	29.7
Labidura riparia	25.4	18.0	27.6	28.9	25.9	19.7	15.8	38.6	37.3	4.4	7.9	50.4	31.1
Anechura bipunctata	21.9	15.8	32.0	30.3	25.4	19.3	16.7	38.6	29.8	9.2	18.9	42.1	37.3
Forficula auricularia[b]	23.7	19.3	30.7	26.3	24.6	20.6	16.2	38.6	21.1	16.7	28.9	44.1	43.4
Forficula lesnei	24.1	18.4	29.4	28.1	24.1	20.2	17.5	38.2	25.9	19.3	27.6	35.1	47.1
Forficula decipiens	24.6	17.1	30.3	28.1	24.1	20.6	18.4	36.8	23.2	13.6	30.3	27.2	32.9

[a] Liu and Beckenbach (1992).
[b] Mijanes-1500 population.

Table 3
Code Bias (%) in First, Second, and Third Codon Positions

	Code Bias[a]		
	First	Second	Third
Insects[b]	17.47	23.20	48.93
Euborellia moesta	12.20	22.00	48.40
Labidura riparia	9.27	19.33	50.27
Anechura bipunctata	16.40	18.67	29.20
Forficula auricularia[c]	9.33	18.13	16.27
Forficula decipiens	11.13	15.80	17.60
Forficula lesnei	10.00	17.20	7.60

[a] Calculated after Irwin, Kocher, and Wilson (1991).
[b] Liu and Beckenbach (1992).
[c] Mijanes-1500 population.

Table 4
Numbers of Transversions (Tv) and Transitions (Ts) in *CO*II between *Euborellia moesta* and Two Other Dermapteran Species

	N	Tv	Ts	Tv/Ts
Positions 1 and 2				
Labidura riparia	71	40	31	1.29
Forficula lesnei	70	31	39	0.79
Position 3				
L. riparia	105	69	36	1.91
F. lesnei	139	66	73	0.90

Note.—Fisher's exact test is nonsignificant between positions for *L. riparia* ($P = 0.136$) and *F. lesnei* ($P = 0.386$), and between lineages for positions 1 and 2 ($P = 0.103$); it is highly significant between lineages for position 3 ($P = 3.3 \times 10^{-3}$).

using Yates' correction, df = 1, $P < 0.01$). The transversion-to-transition ratio (Tv/Ts) tended to decrease at all positions in the *Forficula* branch. This difference was not significant for the first two positions, but it was highly significant ($P < 0.01$) for third positions. This test is very conservative, since substitutions having occurred in the *L. riparia* branch are shared between the two comparisons. A less conservative test can be obtained by estimating the number of transitions and transversions specific to the *L. riparia* lineage and subtracting it from the comparisons shown in table 4. This estimate was obtained from an alignment of *CO*II in these three dermapteran species and in the Orthoptera *L. migratoria.* Nine transversions and two transitions were found only in *L. riparia* for first and second positions. They provided an estimate of substitutions having occurred only in the *L. riparia* lineage, considering homoplasy negligible. These new values provided a less conservative test. However, Fisher's exact test remained nonsignificant for these positions ($P > 0.081$). Consistent results were observed for other forficulids (data not shown). There is thus no evidence that the substitution patterns for first and second positions are different between *Forficulidae* and other insects. All significant effects observed in this data set are entirely due to the third codon positions.

Nature of Substitutions Between Species

It may be interesting to compare the evolution of mtDNA content in earwigs with that in *Drosophila,* for which Clary and Wolstenholme (1985) (see also Garesse 1988) found 93.8% AT- ending codons. By comparing two *Drosophila* species, Clary and Wolstenholme (1985) found that transversions were 4.6 times as frequent as transitions, while A↔T substitutions accounted for 86% of all transversions. Among transitions, C↔T substitutions were more frequent than A↔G substitutions. We can compare these results with those observed between two *Forficula* species, *F. lesnei* and *F. auricularia* (e.g., from the Rome population). In *Forficula,* transitions outnumbered transversions (89 vs. 63). Among transitions, A↔G and C↔T transitions were observed in about equal numbers (47 vs. 42). Only 53.75% of codons ended in A or T.

Directionality of Substitutions Between Species

Another aspect of nucleotide changes is their orientation in favor of a given base. Between *E. moesta,* which is similar to other insects, and *F. lesnei,* 62 of the 73 transitions exchanged either a C for a T (T→C: 31) or a G for an A (A→G: 31) in the direction *E. moesta* → *F. lesnei.* Only 11 changes occurred in the reverse direction (C→T: 4; G→A: 7). A comparison of two Dermaptera showing the same bias in codon usage as other insect orders (in the direction *E. moesta* → *L. riparia*) revealed a more even substitution pattern (C→T: 9; T→C: 8; A→G: 6; G→A: 11).

Directionality of Substitutions Between *F. auricularia* Populations

In a previous paper (Wirth et al. 1998), we showed that the *F. auricularia* taxon is composed of two sibling species, ''A'' and ''B.'' The latter species is itself composed of two vicariant and interfertile groups of populations differing in their reproductive cycles. The two mitochondrial genomes sequenced for *CO*II in *F. auricularia,* Mijanes-1500 and Rome, each belonged to one of these interfertile groups. They differed at 14 positions (including 3 replacements and 11 synonymous substitutions). Of these 14 substitutions, Rome gained 11 CG and 3 AT, whereas Mijanes-1500 gained 2 CG and 12 AT. The difference was significant (Fisher's exact test, $P = 0.00092$). This means that during evolution, the differentiation of the two groups of populations involved an asymmetrical pattern of change.

Phyletic Changes in the Rate of Nucleotide Substitutions

In order to record changes in evolutionary rates in Dermaptera, we carried out a phylogenetic analysis. Trees were rooted using four outgroups: two of them (*Schistocerca gregaria* and *L. migratoria*) were Orthoptera, which are thought to be the insects closest to earwigs. We included one species of Blattaria (*Periplaneta americana*) and an Odonata (*Sympetrum striolatum*). We performed three phylogenetic analyses using the neighbor-joining method (fig. 2). One involved all nucleotides, the second involved only nucleotides from the first and second positions of codons, and the third involved

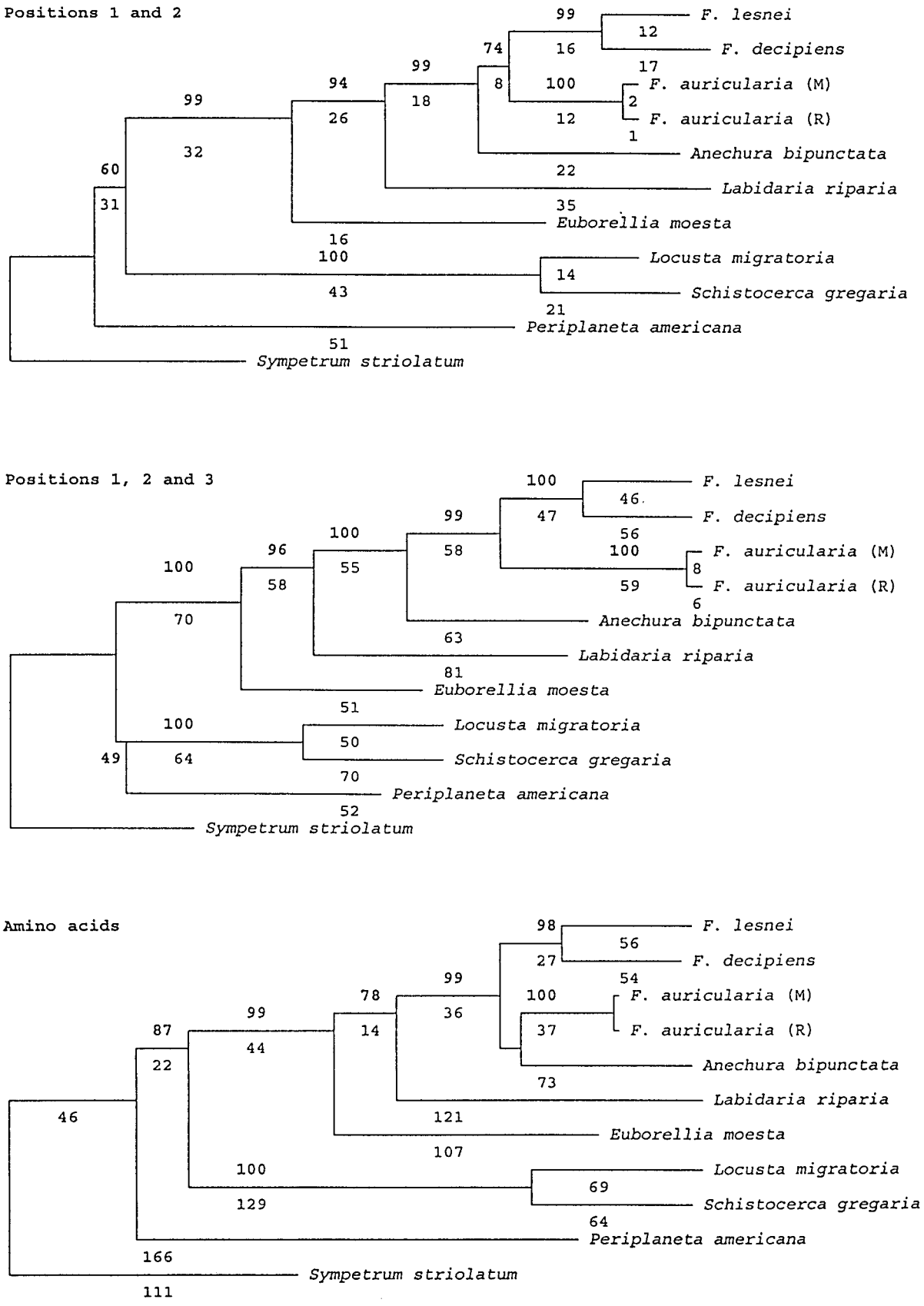

FIG. 2.—Neighbor-joining tree of *COII* sequences from six dermapteran species, using *Locusta migratoria*, *Schistocerca gregaria* (locusts), *Periplaneta americana* (cockroaches), and *Sympetrum striolatum* (dragonflies) as outgroups. Numbers above branches are bootstrap values (in percentages over 1,000 replicates); numbers below branches are the minimum numbers of nucleotide changes per branch, calculated using PAUP. Top: tree based on total nucleotide divergence using Kimura's (1980) two-parameter model; middle: same analysis on first and second codon positions; bottom: tree based on amino acid *p*-distance.

amino acids. In all trees, forficulids appear to be monophyletic with high bootstrap values. The whole group of Dermaptera is also monophyletic, with *L. riparia* always being closer to forficulids than *E. moesta.* Among forficulids, the monophyly of the *Forficula* genus was not confirmed, since *A. bipunctata* occurred within this genus in the amino acid tree. A high bootstrap value separating this species from *Forficula* was found only when all nucleotides were used. The four nondermapteran insects used as outgroups were always at the base of the tree, despite changes and low bootstrap values. As expected, *Sympetrum* was at the root, and Orthoptera were closer to Dermaptera. However, the position of *Periplaneta* was uncertain.

Branch lengths were about equally long in the amino acid tree and the nucleotide tree involving only positions 1 and 2. Those from the tree using all nucleotide positions were very different, with a tendency to longer branches leading to species with larger CG contents. Since the neighbor-joining method relies on a nonintuitive approach to evolutionary distance, we used PAUP, version 3.1.1 (Swofford 1993), to calculate the minimum number of steps involved in nucleotide phylogenies, from the last dermapteran common ancestor downward. *Euborellia moesta* had a small number of changes in all phylogenies. In other species, the number of changes leading to *L. riparia* (61) was close to that leading to *Forficula* (65–85) for positions 1 and 2. When all positions were pooled, the number of changes for *Forficula* (236–274) was about twice that for *L. riparia* (139). This suggests that a strong increase in the substitution rate accompanied the change of bias in the Forficulidae branch.

The lack of variation in the rate of amino acid replacement was interesting, since a change in base content could affect the polypeptide sequence. We therefore compared the COII amino acid sequences of two insect species showing strong AT biases (*D. yakuba* and *A. mellifera*) with those of forficulids, those of nonforficulid Dermaptera, and those of the other insects shown in figure 2, respectively. Differences with *D. yakuba* (ranges, 26.9%–30.0%, 30.0%–30.4%, and 27.8%–33.6%, respectively) and with *A. mellifera* (51.5%–53.8%, 48.8%–53.3%, and 47.0%–52.9%, respectively) were very similar each time and showed no increase in replacement rates with forficulids. The same comparisons were made with a series of vertebrates ranging from coelacanths to humans. Here again, no differences were observed between the three groups of insect taxa (data not shown). The absence of change in amino acid replacement rate accompanying the change in DNA composition is consistent with our observation that the latter mainly involves third codon positions, along with some first codon positions. This suggests that a strong normalizing selection is acting on the polypeptide sequence.

Discussion

Our results show that the *CO*II gene has undergone a dramatic evolution in the Forficulidae. The DNA content at the third codon positions has changed, switching from A-terminating to G-terminating codons, and from T-terminating to C-terminating codons. The first and second codon positions were not significantly affected. This change was accompanied by an increase in nucleotide substitution rate. The CG content, which is highly biased in all other insects, tends to be much less biased in the Forficulidae, and even reaches nonsignificance for third positions in one of the species studied, *F. lesnei.* This evolution appears not to have taken place in other dermapteran families, which are similar in this respect to most insects.

The base content and the pattern of substitutions between all four bases, A, C, G, and T, in the Forficulidae is the opposite of that found in the most extensively studied insect, *Drosophila* (Clary and Wolstenholme 1985; Wolstenholme and Clary 1985). In fruitflies, mtDNA is AT-rich, and transversions strongly outnumber transitions. In *Forficula, CO*II tends to become CG-rich, transitions outnumber transversions, and transitions strongly favor T→C and A→G changes.

In all of these respects, earwigs belonging to two other dermapteran families included in this study, *E. moesta* and *L. riparia,* showed the same pattern as the other insect orders. The evolution of mtDNA in the Forficulidae thus appears to have been both rapid in time and limited in phylogenetic extent.

The rapidity of this evolution had been suggested to us by the study of speciation in the *F. auricularia* species complex (Wirth et al. 1998), for which significant changes in base composition of a 627-bp fragment were recorded between populations from the same species (unpublished data). In the present study, we found a significant bias in substitution pattern between the two lines sequenced from two *F. auricularia* populations.

A high AT content is a feature common to most animal mitochondria. Of 110 taxa surveyed for nucleotide composition of the mitochondrial *Cytochrome b* gene (Jermiin et al. 1994), 109 showed AT pressure, whereas only one species, the bird *Cairana moschata,* showed significant CG pressure. Insects were the animal group showing the greatest directional ''mutation pressure,'' as estimated from equilibrium CG values (0.123; range, 0.052–0.222). The evolution of earwig mtDNA suggests that a simple switch, of an as yet unknown nature, can annihilate these compositional biases.

Several hypotheses have been put forward to explain biases in mtDNA nucleotide contents. One of them is the mutation-based hypothesis, whereby DNA-repairing enzymes tend to create a bias in the frequency of new mutations. Wolstenholme and Clary (1985) explained the mtDNA substitution pattern in *Drosophila* by specificities of enzymes from the transcription and replication apparatus. Proteins that are functionally efficient for an AT-rich DNA may be less optimal for a CG-rich DNA, since the levels of energy required for opening the DNA strands are different. Another mutation-based hypothesis would be that an inversion occurred between the leading strand and the lagging strand of the DNA, which tend to evolve toward different molecular weights in mitochondria. This explanation, how-

ever, is unlikely for earwigs, for which the excess of substitutions occurs among purines, whereas under this hypothesis they would be expected to occur between purines and pyrimidines (Francino and Ochman 1997). Other explanations would be based on selection, which has been shown to act on insect mtDNA (Ballard and Kreitman 1994; Rand et al. 1994).

The actual challenge to molecular evolutionary theories is presented by the strong biases observed in species other than earwigs, such as the 93.8% of A- and T-terminating codons of *CO*II in *D. yakuba.* The loss of bias in *Forficula* could be a return to some equilibrium under relaxed constraints. Evolution has led to the absence of bias in the third positions in *F. lesnei.* We do not know whether this process has finished or whether it will continue and ultimately bias nucleotide content toward high CG levels.

The present paper was not intended to answer these questions. The study of other parts of the earwig mitochondrial genome will be necessary to complete this initial report. The fact that significant changes in compositional bias can be recorded within species suggests that a study of polymorphism would be useful for confronting selective and neutral explanations for the evolution of mtDNA.

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APPENDIX

Alignment of the *COII* Sequence of Six Dermapteran Species with *L. migratoria* (Flook, Rowell, and Gellissen 1995)

The alignment includes two *F. auricularia* populations: 1, from Mijanes; and 2, from Rome.

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Anechura    ATGGCTACATGATCAAATTTAGGGTTTCAGGATAGTGCGGCTCCCCTGATAGAACAATTAACGTTTTTTTCATGATCATACCTTG
F.lesnei    ............G.T.....T........A..C..A.T.T.C...T....G......C.T..C........C..C..C..AC..
Mij15       ........G...G.T..CC.G........A..C.....T..C...T....G.....GC.G..C........C........T...
Rome        ........G...G.T..CC.G........A..C.....T..C...T....G.....GC.G..T........C........T...
F.decip     ............G.T..C..G....C...A..C..A.TAT.C..TA....G..G...C.T..T.....C..C........GA..
Labidura    ........G...G.T....AT...A.A...A....A..C.....AT.A.....G..G....TA........C..C.....AC.A
Euborellia  ...T.......G.....C...A.T.C...A.....A..AT....AT.A.............TA..............A...
Locusta     .....A...........C...TCA..A..A...G.A..TT.A..AT.A............T.A..C..............AA.A

Anechura    TTTATTTTATTAATAATTATTGTAACGGTGGGGTATTTATTAGGAACTTTGTATTTTATTTATTTTATTAATCGGTTTCTATTG
F.lesnei    .....CC.G..G.......CA..G.T.........C.....GA....CC.C.GG....A.CG..A..CA..C..A.........
Mij15       .....C..GC.G.......C.....T...T..A.....G..G..G..CC....C....A.CGG...G.......A.........
Rome        .....C..G..G.......C.....T...T..A.....G..G..G..CC.T..C....A.CGG...G.......A...T.....
F.decip     ........G..G..G....CA..G.T...AA.T......C.GA....CC...GG....A..G..A..CA.....T..CT.G...
Labidura    ......C..G.........C.C...TAA.T......C.T.....TT.....GC.AC..A.C..CA.........T.A...T..A
Euborellia  ......C.T........C.CAA.T.TAA.T.......C.TC.T.TTT..C.T....A..A.A.AC..........C..C..T...
Locusta     A..GA.C.....T....C.CAA...TT..A..A....CGC.TA..TA.A.A.TA..A.CAA.A.A..CA..C..AAA.A..C.T

Anechura    GAGGGGCAAACCATTGAAGTAATTTGAACTATTATCCCAGCGGTGACCTTAGTATTTATTGCGTTACCATCTTTACGGATGCTT
F.lesnei    .....C.G.C.G..GA.............A........T..A.....T...A.T........AC.T..C..A..G..CT..T.G
Mij15       .....T.....T..C..G..TG.......GG.G..T.....T..T..G..GA.T..C.....CC....C..G..G...T..T.G
Rome        .....T.....T..C..G..TG.......GG.G..T..T..T..T..G..GA.T..C.....CC....C..G..G..AT..T.G
F.decip     ..A.....G..G.....G..T.A...G..CG....T..T..A...........T.....C..AC.T..T..A..G..TT..T.G
Labidura    ..A..A.....TG.......T........AG..T.A..T..A..A..A...A.T........T.....T........AT.AT.A
Euborellia  .....A.....A..................G.A..T..G..T..T..T...A.T........C..G.....A......T.AT.A
Locusta     C.T..T..TTTA......ACT..C.....AGCAC.A.....AA.T..A...A.C........A........A.....AT.A..A

Anechura    TATCTTTTAGATGAAGTTAATCAACCTTCTTTGACTTCAAAAGTGGTTGGGCACCAATGGTATTGAAGTTATGAATACTCAGAT
F.lesnei    ..C..G....GC..G...........AG.C..T..C.T......A..G..C..T..............A..C.....T......
Mij15       ..C.............A..........A..CC.T..C.T...G..A..G..T..T..G...........C..C............
Rome        ..C.............A..........A...C.T..C.T...G..A..G..T..T..G...........C..C.....T......
F.decip     ..C..C..G.....G..C.G...G......C.A..G.T...G..A..G..C..T.......T......A..C.....T......
Labidura    .....A.............GA.TT......G.T..C.T......A..A..T..T.....................T......
Euborellia  ...T.A............T..G.C...ATAA.T....T...GA.TA....A..T.....A.....G...........T.....C
Locusta     ...T.AC.T.....TTCATCAG.TG..ATAA.T..AATT...ACAA....A.GA.....A..C.............T......

Anechura    TTCGGGGATGTAGAATTTGACTCATATATAATTCCTGTGGGGGATTTAGGACAAGACGGCTTTCGTTTACTAGAGGTTGATAAT
F.lesnei    ..T...A.C..G.....C..T..C.....GG.A..CTC.........GAA.AT..CA..G.....G..GT.G.....A..C..C
Mij15       ..T........G.....C..T.....C..GG.A..AA.T........G....CT.CG..A..C.....GT.G..A..A..C..C
Rome        ..T........G.....C..T.....C..GG.A..AA.T.....G..G....CT.CG..A..C.....GT.G..A..A..C..C
F.decip     ..T...A.C..G....G......C..C..G.....CTC......C..GA..G.G.CG..G.....GC.GT....A..A..C...
Labidura    ..TAA....A.T..T.....T..C......T.A...C.TA.A......TCTG...GA..A..A...C.TT....A..A..C.T.
Euborellia  ..TAATC.A...........T..T......G....A.AA.AT......A..AT..GT..A.........T....A..G......
Locusta     ...ATTA.............TA.........C.....AAAAT..A...AATAC...T.AA..C..AC.CT....A.........

Anechura    AATGTAGTTTTACCTGTGGGGACTCAAGTTCGCCGTTTAGTAACTGCTGCCGATGTTTTGCATTCTTGAGCTGTACCAGCCTTA
F.lesnei    .....G........CC.TC.T..A.....G..GGC...G..G..A..C........G.....C..A.....A..C.....T..G
Mij15       ..C..T..A..G..C..TC.A..G........GGCG..G..C..A..A.....C...C.T.....G.....G..C..T..A..G
Rome        ..C..T..A..G.....TC.A..G........GGCG..G.....A..A.....C...C.C.....G.....G..C..T..A..G
F.decip     .....G........C..TC.T..G.....G..GGC...G..G..A..A........A..A..C..A.....C..C.....T..G
Labidura    .....T...C.T..G..TTTA.....GA....TGC....A.T..A....GA.....A..A........G........TT.T...
Euborellia  ...A.T.........A.ATTA...........AGC.A.T..T.....G..T........A.....A..G.....T..G..T...
Locusta     CGAAC.ACA......A.AAAT..AG....A..AGTA...ACT.GA..AT.T.....AC.C..C..A.....A........T...

Anechura    GGAGTAAAAGTTGACGCTACCCCAGGGCGATTAAACCAAACTAGTTTTTTTATCAATCGTTCTGGGTTGTTTTACGGGCAATGT
F.lesnei    ..G..T...A.......A..T..C.....G..G...........C............T....A..CA.............G...
Mij15       ..G..T...A....T..C........C..GC....T.............C..T........G...........T.........
Rome        ..G..T...A....T..C........C..G.....T.............C..T........G...........T.........
F.decip     ..G..T...A....T..A.....C.....G.....T.....................T....G...A.......T.........
Labidura    .....C...A....T.....T..T..T..T..G..T.....A...........A.....A..A..C..A.....T..T..G...
Euborellia  ..T......A......GA..T..T.....GC....T....T........ACC.T.....GG....T..A.....T..T..G...
Locusta     .T.T.....A.......A..A..C..A...C........GG..TA...A.A..T..C..CC.A..TC.A....T...T.....C

Anechura    TCTGAGATTTGTGGGGCGAATCATAGTTTTATGCCTTATGTTTTAGAGAGCGTTGCGCCCGAAGGGTTTTTAGGTTGATTAAAA
F.lesnei    ................C................CAT...CC.......A..G..A..G.GG......C.C.AA..G.......
Mij15       ..G..A.....C..A..A..C...........A..CAT....C.......T..GA....A.GGA.T......AA...GC.G..G
Rome        ..G..A.....C..A..A..C...........A..CAT....C.......T..GA....A.GGA.T......AA...GC.G..G
F.decip     ..A..............C.........A.....A...AT....C.......A..G..A..G.GG......C.T.AA..G......
Labidura    ..A..A........T..T.....C...........GAT...AA.T..A..T..AC.TGTAA.TT.A......A.C........G
Euborellia  ..A..A........A..A..................AT..G.A.T..A..A...T.AA.TA.GT.A......AA....G.....
Locusta     ..A..A..C.....A..T........A.....A..AAT...AA.T..A..AACAT.AATTA..CTT..CA.TAAA......TCT

Anechura    AAGGTTAGTTAA
F.lesnei    ....AGC.....
Mij15       ...AAG.CC..G
Rome        ...AAG.CC...
F.decip     .G..GGCA....
Labidura    ..ACAA.C....
Euborellia  .GTT.A---..G
Locusta     ..CA.A.TA...
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