Mitochondrial DNA Sequence of Cytochrome Oxidase II from *Calliphora erythrocephala:* Evolution of Blowflies (Diptera: Calliphoridae)

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ABSTRACT The complementary DNA sequence for the mitochondrially encoded cytochrome oxidase subunit II (COII) gene was determined for the blowfly *Calliphora erythrocephala* Meigen. The sequence has a high A+T content (73%) typical of some insect mitochondrial genomes. The sequence is highly conserved with other Calliphoridae thus far examined, exhibiting between 5.1 and 8.9% nucleotide difference, 3 amino acid substitutions in the body of the protein, and length variation at the carboxy-terminus. Evolutionary analysis using maximum parsimony and maximum likelihood supports the traditional monophyletic grouping of the Calliphoridae and suggests that the Calliphora sequence is more closely related to the blowfly *Phormia regina* Meigen than it is to *Lucillia illustris* Meigen or *Phaenicia sericata* Meigen.

KEY WORDS *Calliphora erythrocephala,* blowflies, *Drosophila,* cytochrome oxidase, mitochondrial DNA, forensics

CYTOCHROME OXIDASE IS a key respiratory enzyme consisting of 3 mitochondrially encoded subunits and at least 4 subunits encoded by the nucleus. The complementary DNA (cDNA) sequence for the cytochrome oxidase subunit II (COII) gene has been determined for many species of bacteria, fungi, plants, and animals (Anderson et al. 1981, Clary and Wolstenholme 1983, Cao et al. 1991). Because of the prevalence of the gene across many taxa, and the high evolutionary rate of the mitochondrially encoded form, the sequence has proven useful for evaluating evolutionary divergence by interspecies comparisons (Adkins and Honeycutt 1994). Recently, the gene sequence has been used to examine evolutionary relationships between many species of insects (Liu and Beckenbach 1992, Beckenbach et al. 1993, Brown et al. 1994) and the usefulness of mitochondrial data for such studies has been reviewed (Simon et al. 1994). The elucidation of COII gene sequences from additional insect species will increase our understanding of these evolutionary relationships.

Sequence data for COII is most useful for evaluating divergence between closely related species. It has been used to measure evolutionary differences between 13 members of the *Drosophila obscura* Fallen species group (Beckenbach et al. 1993). These investigators showed that the *D. affinis* Sturtevant and *D. pseudoobscura* Frolova subgroups are monophyletic groupings that are more closely related to each other than either is to the *D. obscura* subgroup. Analysis of COII sequence data, however, does not allow sufficient resolution between insect species of different orders. Investigations of 10 orders of insects yield different evolutionary relationships depending on the type of analysis used, probably because the divergence is very ancient (Liu and Beckenbach 1992). Furthermore, intraorder comparisons within the Hymenoptera, Coleoptera, and Orthoptera exhibit high sequence divergence that is not sufficient for accurately resolving evolutionary distance (Liu and Beckenbach 1992).

In the Diptera, the family Drosophilidae has been used extensively for evolutionary studies. These investigations have used chromosomal banding patterns (Anderson et al. 1991) mitochondrial restriction fragment polymorphisms (Latorre et al. 1988, Gonzalez et al. 1990), DNA/DNA hybridizations (Caccone et al. 1988, Goddard et al. 1990), and DNA sequence analysis (DeSalle et al. 1987, Beckenbach et al. 1993). The 2 most studied species groups are *D. obscura* and *D. melanogaster* Meigen. The family Calliphoridae is also within the order Diptera, but has not been investigated as extensively as the Drosophilidae. Although several different classification systems for the Diptera are currently used (Hackman and Väisänen 1982), the Calliphoridae and Drosophilidae are often grouped within the division Schizophora. They differ taxonomically in that Calliphoridae are within the series Calyptratae, whereas Drosophilidae are Acalyptratae (McAlpine et al. 1981).

Members of the Calliphoridae, particularly the blowfly genus *Calliphora,* are used routinely for

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comparative studies with flies of the Drosophilidae family. Reported DNA sequence comparisons between *Calliphora* and *Drosophila* include the genes encoding xanthine dehydrogenase (Houde et al. 1989), opsin (Huber et al. 1990), the hunchback protein (Sommer et al. 1992), and the proto-oncogene *abl* (Durica et al. 1987). The divergence between the Drosophilidae and Calliphoridae has been estimated at 99 million years based on the rate of evolutionary change in a larval hemolymph protein (Beverley and Wilson 1984).

To examine further the structure of the mitochondrially encoded COII gene, and to investigate the evolutionary relationships between different species within the Calliphoridae, we have sequenced the cDNA for COII from *C. erythrocephala* Meigen. We present the cDNA sequence, predicted amino acid sequence for the protein, and analyze the degree of divergence between different members within the Calliphoridae family.

Materials and Methods

Complimentary DNA Library Screening and cDNA Subcloning. A *C. erythrocephala* retina cDNA library (Huber et al. 1990), was used to infect *Escherichia coli* strain XL1Blue and plated. Hybond membranes (Amersham, Arlington Heights, IL) were used for plaque lifts following manufacturer protocol. The filters were hybridized to ^{32}P-labeled DNA corresponding to a cross-hybridizing *D. melanogaster* probe. Hybridization and wash conditions were as described in Heberlein and Rubin (1990). Approximately 15,000 plaques were screened. Cross-hybridizing clones were picked and plaque purified as described in Sambrook et al. (1989). Candidate COII molecules in pBluescript plasmids were excised in vivo following manufacturer protocol (Stratagene, La Jolla, CA).

Sequencing of DNA. Both strands of the COII cDNA in Bluescript were sequenced using the M13-20 primer, and Reverse primers. Additional primers corresponding to internal cDNA sequence were purchased from Oligo's Etc. (Wilsonville, OR). Templates were sequenced using the Sequenase II kit from United States Biochemical (USB) Corporation according to manufacturer protocol.

Data Analysis. DNA sequences were conceptually translated using the MacVector program for the Macintosh (Eastman Kodak, Rochester, NY), and the *Drosophila* mitochondrial code (de Bruijn 1983). Sequences were aligned by the Assembly LIGN program (Eastman Kodak, Rochester, NY) following manufacterer protocol. Phylogenetic analysis was accomplished using the PHYLIP package version 3.5c of Felsenstein (1993). Unrooted parsimony analysis was performed using PHYLIP DNAPARS 3.54c. Distances were computed using the PHYLIP DNADIST 3.54c program to generate pairwise corrected haplotype distances, using Kimura's (1980) 2 parameter model (transitions to transversion ratios from 0.0 to 8.0 were used). Distance trees were constructed by the neighbor-joining method (Saitou and Nei 1987), using the PHYLIP NEIGHBOR 3.5c program. Distance matrix bootstrapping was done using DNADIST, NEIGHBOR, and SEQBOOT of the PHYLIP 3.5c package. We also used the maximum likelihood program, PHYLIP DNAML 3.54c, as an additional phylogenetic approach.

Results

Nucleotide Sequence and Predicted Amino Acid Sequence of *C. erythrocephala* Cytochrome Oxidase II. The 640 nucleotide *C. erythrocephala* cDNA sequence and predicted amino acid sequence (Fig. 1) has several characteristics indicating that it is cytochrome oxidase subunit II and is mitochondrially encoded. Genbank searches using this sequence identified COII from over 30 different species. The DNA sequence is 73% A+T, not including the poly (A+) tail, exhibiting a high A+T ratio. The mitochondrial genetic code for *D. melanogaster* mitochondria (de Bruijn 1983) was used to infer the corresponding amino acid sequence. The codon UGA appears 5 times in the *C. erythrocephala* sequence. In the nucleus UGA is a stop codon, but in the *D. melanogaster* mitochondria it codes for tryptophan. Because only this nuclear stop codon appears in the *C. erythrocephala* reading frame at the appropriate location corresponding tryptophan, it strongly suggests that the cDNA is mitochondrially encoded.

Comparison of *Calliphora* Amino Acid Sequences. A comparison of the predicted *C. erythrocephala* COII amino acid sequence to other published Calliphoridae species (Sperling et al. 1994) demonstrates a high degree of homology and supports the identity of functional regions of the protein by conservation (data not shown). The alignment demonstrates that the *C. erythrocephala* molecule is truncated such that 19 N-terminal amino acids are missing, suggesting that this cDNA is not full length. Conserved in the *C. erythrocephala* sequence is the aromatic region between amino acids 101 and 113, which is involved in electron transport between copper and heme (Millet et al. 1983). The putative copper binding sites including His^{161} and the amino acids from position 192 to 202 are conserved, as are the 3 amino acids believed to be involved in binding cytochrome c at Asp^{111}, Asp^{158}, and Glu^{198}.

Sequence comparison shows 3 variable amino acid sites within the body of the protein, and considerable variation at the carboxy terminus. The 3 internal variations consist of 2 conservative isoleucine/valine replacements and 1 alanine/threonine replacement at position 114. The amino acid alanine/threonine position 114 is highly variable between insect species (Liu and Beckenbach 1992); members of the genus *Drosophila* typically have

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        10         20         30         40         50         60         70         80
                                                     *
ATTAGTCTTT TTTCATGACC ACGCACTTTT AATTTTAGTA ATAATTACTG TTCTAGTAGG ATACTTAATA TTTATACTAT
 LeuValPhe PheHisAspH isAlaLeuLe uIleLeuVal MetIleThrV alLeuValGl yTyrLeuMet PheMetLeuP

        90        100        110        120        130        140        150        160
                   *                                                        *
TTTTTAACAA ATATGTAAAT CGATACTTAC TTCATGGACA AACTATTGAA ATTATTTGAA CAATTTTACC TGCAATTATT
hePheAsnLy sTyrValAsn ArgTyrLeuL euHisGlyGl nThrIleGlu IleIleTrpT hrIleLeuPr oAlaIleIle

       170        180        190        200        210        220        230        240
                                        *
TTACTATTTA TTGCATTTCC TTCTCTTCGA CTTTTATACT TATTAGATGA AATTAATGAA CCTTCTATTA CTTTAAAGGC
LeuLeuPheI leAlaPhePr oSerLeuArg LeuLeuTyrL euLeuAspGl uIleAsnGlu ProSerIleT hrLeuLysAl

       250        260        270        280        290        300        310        320
         *                                                      *
AATTGGACAT CAATGATATT GAAGTTATGA ATATTCAGAC TTTGCAAATA TTGAATTTGA TTCATATATG ATTCCTACTA
aIleGlyHis GlnTrpTyrT rpSerTyrGl uTyrSerAsp PheAlaAsnI leGluPheAs pSerTyrMet IleProThrA

       330        340        350        360        370        380        390        400
                             *                                                        *
ATGAATTATC AATTGATAGT TTTCGTCTAT TAGACGTTGA TAATCGAGTA GTCTTACCAA TAAATTCTCA AATCCGAATT
snGluLeuSe rIleAspSer PheArgLeuL euAspValAs pAsnArgVal ValLeuProM etAsnSerGl nIleArgIle

       410        420        430        440        450        460        470        480
                                                     *
TTAGTAACTG CTGCAGATGT AATTCATTCT TGAACTATTC CAGCTTTAGG AGTAAAGGTA GATGGAACTC CTGGTCGATT
LeuValThrA laAlaAspVa lIleHisSer TrpThrIleP roAlaLeuGl yValLysVal AspGlyThrP roGlyArgLe

       490        500        510        520        530        540        550        560
                   *                                                        *
AAATCAAACA AACTTTTTAA TTAACCGACC TGGTTTATTT TATGGACAAT GTTCAGAAAT TTGTGGAGCT AATCATAGTT
uAsnGlnThr AsnPheLeuI leAsnArgPr oGlyLeuPhe TyrGlyGlnC ysSerGluIl eCysGlyAla AsnHisSerP

       570        580        590        600        610        620        630        640
                                        *
TTATACCAAT TGTAATTGAA AGAATCCCAG TAAATTATTT TATTAAATGA ATTTCTAGCA TAAACTCTTA AAAAAAAAAA
heMetProIl eValIleGlu SerIleProV alAsnTyrPh eIleLysTrp IleSerSerM etAsnSer** *
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Fig. 1. *C. erythrocephala* cytochrome oxidase subunit II (COII); cDNA sequence and predicted protein. The nucleotide sequence of the COII gene is shown. The predicted amino-acid sequence is shown below the nucleotide sequence.

asparagine (Beckenbach et al. 1993). The predicted *C. erythrocephala* protein sequence is 227 amino acids long, compared with 231 for the other *Calliphora* species. The length variation is the result of differences at the carboxy terminus.

Comparison of *Calliphora* COII DNA Sequences. The DNA sequences of *Calliphora* species presented in Fig. 2 are aligned for 692 bp and demonstrate more variability than the amino acid sequences. Because of the difficulty in aligning the sequences at the 3′ end of the insect gene, comparisons of insect COII have typically ignored the carboxy-terminus (Liu and Beckenbach 1992). We do not use sequences 3′ to position 615 of the *C. erythrocephala* sequence in the analysis that follows. The comparisons are based in part upon previously reported sequences of Sperling et al. (1994).

Pairwise comparisons of nucleotide differences between representative insect COII DNA sequences are summarized in Table 1. The corrected divergences (Kimura 1980) were calculated for all combinations of species (Table 1, below the diagonal). Species within the Calliphoridae family are the most similar. For these, the total substitutions per 100 nucleotides ranged from 5.1 to 8.98%. The most similar species are *Lucillia illustris* Meigen and *Phaenicia sericata* Meigen. Members of the Calliphoridae family are most similar to the Drosophilidae family. The Calliphoridae exhibited between 14.15 and 15.99% sequence difference from *D. yakuba* Burla. The Calliphoridae and Droso-

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C. erythrocephala ----------------------------------------------------ATTA   4
P. sericata       ATGTCAACATGAGCAAATTTAGGTTTACAAGATAGTTCTTCTCCTTTAATAGAACA....  60
L. illustris      ..............................................................  60
P. regina         ..............................................................  60

C. erythrocephala GTCTTTTTTCATGACCACGCACTTTTAATTTTAGTAATAATTACTGTTCTAGTAGGATAC  64
P. sericata       A.............T.................................A..T.........  120
L. illustris      A..........C..T.....................................T........T  120
P. regina         A................T.....................................T..T  120

C. erythrocephala TTAATATTTATACTATTTTTTAACAAATATGTAAATCGATACTTACTTCATGGACAAACT  124
P. sericata       .....G......T.............................T...T.A..C.........  180
L. illustris      ............T.............................T...T.A............  180
P. regina         ....................C.....................................  180

C. erythrocephala ATTGAAATTATTTGAACAATTTTACCTGCAATTATTTTACTATTTATTGCATTTCCTTCT  184
P. sericata       ..................................T...........T..C......  240
L. illustris      ............................A.............T..........T.......  240
P. regina         ..................................T...........T.........  240

C. erythrocephala CTTCGACTTTTATACTTATTAGATGAAATTAATGAACCTTCTATTACTTTAAAGGCAATT  244
P. sericata       ..............T...C.T.....................A..................  300
L. illustris      ......T.A.........C.T.....................A..................  300
P. regina         ..A.....................................A.....C............  300

C. erythrocephala GGACATCAATGATATTGAAGTTATGAATATTCAGACTTTGCAAATATTGAATTTGATTCA  304
P. sericata       ..T.................................T..................C......  360
L. illustris      ..............C.........................A...................  360
P. regina         .....C........C.....................T.....................T  360

C. erythrocephala TATATGATTCCTACTAATGAATTATCAATTGATAGTTTTCGTCTATTAGACGTTGATAAT  364
P. sericata       .....A...........C.................C......T.......T..A......  420
L. illustris      ..C..A........A.....................A..C...T................C  420
P. regina         .....A........A.......................C...T..................  420

C. erythrocephala CGAGTAGTCTTACCAATAAATTCTCAAATCCGAATTTTAGTAACTGCTGCAGATGTAATT  424
P. sericata       ........T...................G.T.....................T.........  480
L. illustris      ........T.....................T..................C............  480
P. regina         ........T..............A.....T..............A..A..T.........  480

C. erythrocephala CATTCTTGAACTATTCCAGCTTTAGGAGTAAAGGTAGATGGAACTCCTGGTCGATTAAAT  484
P. sericata       ..............C.....AC................................C.....  540
L. illustris      ..............C.....A.....................T............C....C  540
P. regina         .....A........C..............T...........T..A..C..A........C  540

C. erythrocephala CAAACAAACTTTTTAATTAACCGACCTGGTTTATTTTATGGACAATGTTCAGAAATTTGT  544
P. sericata       ........T..................A...........C.....................  600
L. illustris      ........T...........T..................C.....................  600
P. regina         ......................T.....A.................................  600

C. erythrocephala GGAGCTAATCATAGTTTTATACCAATTGTAATTGAAAGAATCCCAGTAAATTATTTTATT  604
P. sericata       ..........................................T...........C......  660
L. illustris      ..........................................T...........C......  660
P. regina         ......................G.....................T...........C.....C  660
                                         ↓
C. erythrocephala AAATGAATTTCTAG-CA--TAAACTCT*        628
P. sericata       ..G..........ATA.TA....T...TCATT*   692
L. illustris      ..G..........ATA.TA....T...TCATT*   692
P. regina         ............ATA.TG........TCATT*   692
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Fig. 2. Sequence comparison of the mitochondrial COII genes of 4 species of the Calliphoridae family. The species *C. erythrocephala* is compared with the sequences reported by Sperling et al. (1994) of *P. sericata, L. illustris,* and *P. regina.* (.) The same nucleotide is present as that in *C erythrocephala,* and (-) the sequence has not been determined at that position.

Table 1. Interspecific divergence in COII DNA sequences in the Insecta

Species	*P. sericata*	*L. illustris*	*P. regina*	*C. erythrocephala*	*D. yakuba*	*A. gambiae*	*S. vittatum*
P. sericata		63.33	64.00	72.50	NC	NC	NC
L. illustris	5.10		72.50	72.50	NC	NC	NC
P. regina	8.98	7.39		56.76	NC	NC	NC
C. erythrocephala	7.38	7.38	6.72		50.00	NC	NC
D. yakuba	15.15	14.15	15.17	15.99		NC	NC
A. gambiae	21.31	20.51	19.22	22.28	24.21		NC
S. vittatum	22.89	21.13	22.44	21.16	20.50	21.01	
C. rosaceana	22.79	22.45	23.92	23.85	23.04	24.98	23.19

Differences/100 bases are shown below the diagonal and are corrected as described (Kimura 1980). Transitions/100 substitutions are shown above the diagonal. Transitions/100 substitutions were not calculated for the outgroup *C. rosaceana*. NC, values not calculated are abbreviated.

philidae families, both of which are within the suborder Brachycera, are more similar to each other than they are to the representatives of the suborder Nematocera, *Anopheles gambiae* Giles and *Simulium vittatum* Zetterstedt. The Brachycera exhibit between 19.22 and 24.21% divergence from the Nematocera. The sequence difference between distantly related members of the same order is similar in magnitude to the sequence difference between members of different orders. For example, all of the representatives of the order Diptera studied here differed from the order Lepidoptera by between 22.45 and 24.98%.

A common characteristic of very similar mitochondrial DNA sequences is that they show a high transition bias (Brown 1985). We also find transition bias when comparisons are made between the members of the Calliphoridae. In these comparisons (Table 1), the transitions predominate, ranging from 56.76 to 72.5% of the substitutions. When *C. erythrocephala* is compared with more divergent species such as *D. yakuba,* the transition bias is essentially eliminated. Interestingly, not all similar sequences demonstrate this high transition bias. *D. melanogaster* and *D. yakuba* exhibit a sequence difference of only 7.2%, and yet transversions outnumber transitions 50.9–49.1% (Wolstenholme and Clary 1985).

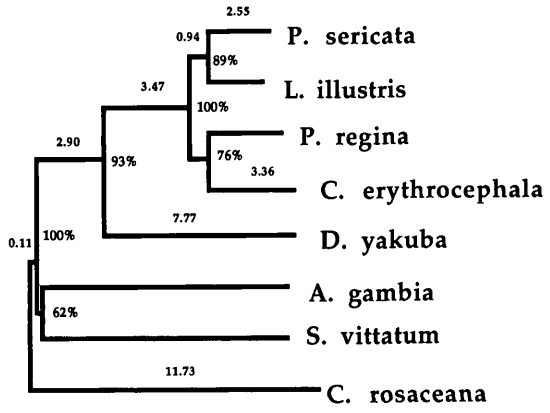

Fig. 3. Distance tree from nucleic acid differences. Shown is the most parsimonious tree of mtDNA sequences using the distance shown in Table 1 and the Kimura model (1980) with equal weighting of all nucleotide substitutions. The Lepidoptera species *C. rosaceana* Harris, accession number L19099 (Sperling and Hickey 1994) is specified as an outgroup. Branch lengths are proportional to the number of changes. Bootstrap percentages for nodes are indicated. Sequences used also include *D. yakuba,* accession number X00924 (Clary and Wolstenholme 1983); *L. illustris,* accession number L14945; *P. regina,* accession number L14946; *P. sericata,* accession number L14947; (Sperling et al. 1994); *A. gambiae,* accession number L20934 (Beard et al. 1993); and *S vittatum* (X. Zhu, K. Pruess, and T. Powers, Genbank accession number M76433).

Evolutionary Relationships Between the Calliphora Family and Representative Insects. Evolutionary constraints of sequences are important to examine before attempting phylogenetic analysis. Only 1.3% of the nucleotide changes among the Calliphora led to amino acid replacements; thus, all but 3 of the nucleotide changes are silent. In total, 88.5% of the nucleotide sites were invariable among the Calliphoridae in the regions analyzed. In total, 71 (11.5%) of the 615 nucleotide sites, representing 67 (29.9%) of the 224 codons, are variable. We find the A+T content at the 1st position of the codon is 59.33, the 2nd is 68.43, and the 3rd is 90.9%. The 3rd position in the codon showed the highest variability, representing 83.6% of the changes. First position changes represented 10.4% of the total, and 6.0% were 1st and 3rd position changes. No substitutions were found at the 2nd position. The 3 amino acid replacements are caused by transitions at the 1st position of the codon.

Phylogenetic relationships among the Calliphoridae and other representative insects were examined by computer analysis using the PHYLIP 3.5c package of Felsenstein (1993). Unrooted parsimony analysis, with PHYLIP DNAPARS 3.5c, yielded 1 most parsimonious tree with the same topology as the tree shown in Fig. 3. Distances were determined with the PHYLIP DNADIST 3.54c program using Kimura's 2-parameter model (1980) and the transition/transversion ratio equal to 2. These distances are shown in Table 1 as total nucleic acid substitutions (lower diagonal). Different distance models in the DNADIST program,

using Jukes and Cantor (1969), or maximum parsimony (Felsenstein 1993), yield similar distances (data not shown). Similarly, distance modeling with differential character weighting, ignoring the 3rd position of the codon and using the neighbor-joining analysis, yielded the same tree with only slightly different distances. Second codon character weighting was not used because there are no differences at these positions in the Calliphoridae species studied. Distance trees were constructed in several ways. Distance trees constructed with PHYLIP 3.54c DNADIST using the neighbor joining method (Saitou and Nei 1987), or with the unweighted pair group with arithmetic means (UPGMA) method gave identical topologies, with slightly different distances. The neighbor-joining tree computed with the distances shown in Table 1 using the Kimura model (1980) is shown in Fig. 3.

As an alternative phylogenetic approach, we used the maximum likelihood program (PHYLIP DNAML 3.54c) program. Maximum likelihood analysis attempts to identify trees with the highest likelihood given a probabilistic model of sequence evolution. We used the empirically determined frequencies of nucleotides and an average transition/transversion ratio determined by pairwise comparisons of all taxa in the analysis. The options we used included the jumble and the global rearrangements to increase the possibility that the tree with the greatest likelihood was uncovered. The tree determined by this method has the same topology as the one shown in Fig. 3, with slightly different distances.

The branch points are supported by the bootstrap parsimony analysis. Features of the relationships depicted in the tree correspond very well to prior classifications of these species. The 2 species *S. vittatum* and *A. gambia* of the suborder Nematocera, were a monophyletic group. Families within the suborder Brachycera, the Calliphoridae, and Drosophilidae, grouped together in 93% of the 100 bootstrapped samples. The Calliphoridae family clusters as a monophyletic group as supported by 100% of the bootstrapped samples. Within the Calliphoridae, *Phormia regina* Meigen and *C. erythrocephala* grouped together, and *L. illustris* and *P. sericata* grouped together.

Discussion

The COII mitochondrial gene sequence is valuable for deriving phylogenetic relationships. The sequence has been determined for many species, providing an extensive data base for detailed comparisons. Because the evolution of mitochondrial genes are subject to different selection pressures than morphological features, the derivation of phylogenetic histories using molecular variation can complement histories derived from traditional studies of morphological characteristics.

Our study confirms and extends the discovery of a high A+T content for some insect mitochondrial genes, particularly at the 3rd position of the codon (Clary and Wolstenholme 1983, Liu and Beckenbach 1992, Brown et al. 1994). The lower A+T content for the 1st and 2nd codons may be the result of the greater constraints that these have on the amino acid composition of the encoded protein (Wolstenholme and Clary 1985).

The analysis of insect COII sequence data is somewhat simplified because of the apparent lack of significant intraspecific variation (Beckenbach et al. 1993, Sperling et al. 1994). The determination of phylogenetic histories using molecular data is complicated however by the many different approaches to statistical examination of sequence variation. Furthermore, there is no consensus as to which method is the most accurate for reconstructing phylogeny. Studies of COII sequence variation in *D. obscura* using transversion distances and 2nd codon distances demonstrate that these types of analysis can be useful for resolving biologically meaningful groupings (Beckenbach et al. 1993). In contrast, character or substitution weighting in studies of COII sequence data in moth species did not substantially alter their inferences from those based on equal weighting (Brown et al. 1994). We found that maximum parsimony analysis, with or without character weighting, was sufficient for grouping the families tested within the Diptera.

With the determination of COII sequence data from many insect species, it is now possible to further compare sequence differences within orders. Previous studies have shown sequence divergences of 37, 38, and 45% within the Hymenoptera, Orthoptera, and Coleoptera orders, respectively (Liu and Beckenbach 1992). We see a smaller intraorder sequence difference within the Diptera. Predictably, the greatest divergence is seen between representatives of the most morphologically distant divisions. The suborders Brachycera and Nematocera are the most divergent and exhibit sequence differences ranging between 19.22 and 24.21%. Representative species within the Brachycera exhibit smaller differences.

The relationships between species within the Calliphoridae family as determined from traditional morphological studies, is similar to the relationships as determined by COII sequence data. Both methods closely relate *L. illustris* and *P. sericata.* As shown in Fig. 4., these species have been grouped in the subfamily Calliphorinae, and the tribe Luciliini (McAlpine et al. 1981). The distance matrix of COII sequence data shown in Fig. 3 supports this close relationship. This classification system also groups *erythrocephala* in the same subfamily as *L. illustris* and *P. sericata,* but in a different tribe, the Calliphorini. Our maximum parsimony analysis of the nucleotide sequence data however, groups *C. erythrocephala* more closely to *P. regina* than to other subfamily members. In a system proposed by Rognes (Rognes 1991), *C. erythrocephala* and *P. regina* are grouped in the separate subfamilies Calliphorinae and Chrysomyi-

A. Classification System for the Order Diptera: (From McAlpine et al., 1981)

Suborder	Infraorder	Division	Series	Superfamily	Family
Nematocera					
Brachycera:	Tabanomorpha				
	Asilomorpha				
	Muscomorpha:	Aschiza			
		Schizophora:	Acalyptratae		
			Calyptratae:	Muscoidea	
				Hippoboscoidea	
				Oestroidea:	Oestridae
					Sarcophagidae
					Rhinophoridae
					Tachinidae
					Calliphoridae

B. Classification Systems for the Family Calliphoridae:

(From McAlpine et al., 1981)			(From Rognes, 1991)	
Subfamily	**Tribe**	**Genus**	**Subfamily**	**Genus**
Chrysomyiinae:	Chrysomyiini		Chrysomyinae:	Protocalliphora
	Phormiini:	Protocalliphora		*Phormia*
		Phormia		Protophormia
		Boreellus		Trypocalliphora
		Protophormia		
		Rhiniini		
Calliphorinae:	Polleniini		Polleniinae	
	Angioneurini		Melanomyinae	
	Luciliini:	*Lucilia*	Luciliinae:	*Lucilia*
		Phaenicia	Rhinophorinae	
		Francilia	Helicoboscinae	
		Bufolucilia	Rhiniinae	
	Calliphorini:	Bellardia	Calliphorinae:	Bellardia
		Cyanus		*Calliphora*
		Acrophaga		Cynomya
		Cynomya		Onesia
		Aldrichina		
		Acronesia		
		Eucalliphora		
		Calliphora		

Fig. 4. Classification systems for the order Diptera and the family Caliphoridae. (A) Partial system as derived from McAlpine et al. (1981) for the Diptera. (B) Two systems for the family Calliphoridae as derived from McAlpine et al. 1981 and Rognes 1991. Only the groupings with species examined in this study are shown.

inae. Although it is clear that more data is needed for resolving these classification systems, our data supports the phylogeny proposed by Rognes where *C. erythrocephala* is not as closely related to the Luciliini tribe.

Calliphora is often used in comparative studies with *D. melanogaster.* These studies are useful for investigating the degree of diversity within the Diptera. Furthermore, because *Calliphora* flies are much larger than *Drosophila,* it is simple to obtain larger quantities of a specific tissue. This facilitates biochemical analysis in *Calliphora* which can complement genetic analysis in *Drosophila.* Of *Calliphora* sequences examined to date, most have a high degree of homology to *Drosophila.* The major opsin of *C. erythrocephala* RH1 has a 14% amino acid sequence difference (Huber et al. 1990) from *D. melanogaster.* The *abl* gene sequences are 21% different (Durica et al. 1987). The *C. vicina* Robineau-Desvoidy xanthine dehydrogenase is 24% different in amino acid sequence from *D. melanogaster* (Houde et al. 1989). The mtDNA sequence for the *C. erythrocephala* COII, reported here, differs 16% from *D. melanogaster.*

In addition to providing information for structural comparisons of the protein and evolutionary analysis, the *C. erythrocephala* sequence can also be used in forensic studies. The precise identifi-

cation of blowfly larvae discovered on a corpse can be used for estimating the time of death. Calliphoridae family DNA sequences for the COII gene have been reported as markers for insect identification by polymerase chain reaction amplification and sequencing (Sperling et al. 1994). The cDNA sequence presented here for *C. erythrocephala* can be used in conjunction with these previously reported sequences to distinguish between blowfly species.

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