The Highly Rearranged Mitochondrial Genome of the Plague Thrips, *Thrips imaginis* (Insecta: Thysanoptera): Convergence of Two Novel Gene Boundaries and an Extraordinary Arrangement of rRNA Genes

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To help understand the mechanisms of gene rearrangement in the mitochondrial (mt) genomes of hemipteroid insects, we sequenced the mt genome of the plague thrips, *Thrips imaginis* (Thysanoptera). This genome is circular, 15,407 bp long, and has many unusual features, including (1) rRNA genes inverted and distant from one another, (2) an extra gene for tRNA-Ser, (3) a tRNA-Val lacking a D-arm, (4) two pseudo-rRNA genes, (5) duplicate control regions, and (6) translocations and/or inversions of 24 of the 37 genes. The mechanism of rRNA gene transcription in *T. imaginis* may be different from that of other arthropods since the two rRNA genes have inverted and are distant from one another. Further, the rRNA genes are not adjacent or even close to either of the two control regions. Tandem duplication and deletion is a plausible model for the evolution of duplicate control regions and for the gene translocations, but intramitochondrial recombination may account for the gene inversions in *T. imaginis*. All the 18 genes between control regions #1 and #2 have translocated and/or inverted, whereas only six of the 20 genes outside this region have translocated and/or inverted. Moreover, the extra tRNA gene and the two pseudo-rRNA genes are either in this region or immediately adjacent to one of the control regions. These observations suggest that tandem duplication and deletion may be facilitated by the duplicate control regions and may have occurred a number of times in the lineage leading to *T. imaginis*. *T. imaginis* shares two novel gene boundaries with a lepidoposcid species from another order of hemipteroid insects, the Psocoptera. The evidence available suggests that these shared gene boundaries evolved by convergence and thus are not informative for the interordinal phylogeny of hemipteroid insects. We discuss the potential of hemipteroid insects as a model system for studies of the evolution of animal mt genomes and outline some fundamental questions that may be addressed with this system.

Introduction

Rearrangements of mitochondrial (mt) genes are proposed to be powerful phylogenetic markers (Macey et al. 1997; Boore and Brown 1998; Boore, Lavrov, and Brown 1998; Morrison et al. 2002). However, there are arguments that these markers should be used with caution since the mechanisms of mt gene rearrangement are still poorly understood (Mindell, Sorenson, and Dimcheff 1997; Boore and Brown 1998; Boore, Lavrov, and Brown 2001; Shao et al. 2001). The abundance of gene rearrangements in the mt genomes of hemipteroid insects, Phthiraptera (lice), Psocoptera (psocids, book lice and bark lice), and Thysanoptera (thrips) have rearrangements of protein-coding genes and tRNA genes relative to the putative ancestral arrangement (Shao et al. 2001). Moreover, the gene arrangements differ among these orders (Shao et al. 2001). However, all species from the fourth hemipteroid order, the Hemiptera (bugs, cicadas, aphids, and kin), have the putative ancestral gene arrangement of hexapods (Dotson and Beard 2001; Shao et al. 2001).

In contrast to other hexapods, all species studied from three of the four hemipteroid orders, Phthiraptera (lice), Psocoptera (psocids, book lice and bark lice), and Thysanoptera (thrips) have rearrangements of protein-coding genes and tRNA genes relative to the putative ancestral arrangement (Shao, Campbell, and Barker 2001; Shao et al. 2001). Moreover, the gene arrangements differ among these orders (Shao et al. 2001). However, all species from the fourth hemipteroid order, the Hemiptera (bugs, cicadas, aphids, and kin), have the putative ancestral gene arrangement of hexapods (Dotson and Beard 2001; Shao et al. 2001).

The abundance of gene rearrangements in the Phthiraptera, Psocoptera, and Thysanoptera, and the ancestral gene arrangement in the Hemiptera, make the hemipteroid assemblage an ideal group for studies of
Highly Rearranged Mitochondrial Genome of the Plague Thrips

FIG. 1.—The mitochondrial genome of Thrips imaginis. DNA strands are shown as two thick-line circles. The two curved thin lines with arrows and numbers at each end show the long-PCR–amplified fragments. The arrows indicate the direction of PCR amplification and the numbers indicate the position of the 5′ ends of the PCR primers. Genes are represented as boxes. Arrows inside or outside boxes adjacent to the inner circle indicate the direction of transcription. Abbreviations of protein-coding and rRNA genes are defined in the text. tRNA genes are named with single-letter amino acid abbreviations except for those coding for leucine and serine, which are named as L1 (anticodon tag), L2 (taa), S1 (tct), S2 (tga), and S3 (gct). CR is the abbreviation for the control region.

the evolution of animal mt genomes. Entire mtDNA sequences are desirable for such studies. However, only two hemipteroid insects, Heterodoxus macropus (Phthiraptera) (Shao, Campbell, and Barker 2001) and Triatoma dimidiata (Hemiptera) (Dotson and Beard 2001) have been sequenced for entire mt genome. For the other two hemipteroid orders, Thysanoptera and Pscoptera, only partial mtDNA sequences are available (Shao et al. 2001).

Here, we present the entire nucleotide sequence of the mt genome of the plague thrips, Thrips imaginis (Thysanoptera). This genome is highly rearranged and has many unusual features. T. imaginis is the first known species of arthropod which has inverted and distantly separated rRNA genes. Further, T. imaginis is the first known species of animal which has both duplicate control regions and rearrangements of most mt genes. We discuss the possible mechanisms of tRNA gene transcription, evolution of duplicate control regions, and gene rearrangements in T. imaginis. We evaluate the phylogenetic value of two novel gene boundaries shared by T. imaginis (Thysanoptera) and a lepidopsocid species (Pscoptera). Finally, we discuss the potential of the hemipteroid assembly as a model system for studies of the evolution of animal mt genomes and outline some fundamental questions, which may be addressed with this system.

Materials and Methods

Collection of T. imaginis and Long-PCR Amplification

Specimens of T. imaginis were collected in Brisbane, Queensland, Australia and were identified in the Department of Zoology and Entomology, The University of Queensland. Live thrips were snap frozen in liquid nitrogen and stored at −70°C. Total DNA was extracted from one thrips with DNeasy Tissue Kit (QIAGEN). The entire mt genome of T. imaginis was amplified in two overlapping fragments by long-PCR amplification with the following primers: C1-J-5408 (5′-ggaggttggagatgttg-3′) with C3-N-8677 (5′-tcaagagttcgcgctta-3′) and C3-J-8290 (5′-caggaattgaaataa-3′) with C1-N-5865 (5′-aatggttggctccctctccgctggtaggacaaag-3′) (fig. 1). The names of the primers indicate the target gene (C1 for cox1 and C3 for cox3), the strand (J for majority and N for minority), and the 5′ base position in the mt genome of T. imaginis. The two fragments were 3,270 bp and 12,983 bp long and overlapped by 388 bp of cox3 and 458 bp of cox1. ELONGASE Enzyme Mix (GIBCO BRL®) was used in long-PCR amplification. The cycling conditions were 1 min at 94°C followed by 35 to 40 cycles of 30 s at 94°C, 30 s at 50°C, and 4 to 15 min at 68°C, and then 10 to 20 min at 68°C.

Sequencing and Cloning

Long-PCR fragments were purified with the QIAquick PCR Purification Kit (QIAGEN). The entire 3,270-bp fragment was sequenced directly with the PCR primers and internal primers. However, the 12,983-bp fragment was sequenced in a different way. First, this fragment was sequenced directly with the PCR primers and a set of conserved primers. Second, the primers which worked in the first step were used in the second-round of PCR to enrich sequencing templates. Second-round PCR fragments were purified and sequenced directly. Third, the pGEM®-T Easy Vector System (Promega) was used to clone four second-round PCR fragments (0.6 to 2 kb) which failed to be sequenced directly. Colones that contained the four PCR fragments were selected and the plasmids were isolated with the High Pure Plasmid Isolation Kit (Roche Molecular Biochemicals). These plasmids were then sequenced with M13 primers and internal primers. The BigDye™ Terminator Kit (Applied Biosystems) was used in sequencing reactions. Sequencing products were resolved by an ABI 377 sequencer. The internal primers were designed to ensure that the nucleotide sequences from two neighboring sequencing reactions overlapped by at least 100 bp. The nucleotide sequences of both strands were determined for the entire mt genome (see GenBank accession number AF335993).

Analysis of Nucleotide Sequence

Nucleotide sequences were aligned with Sequencher™ (Gene Codes Corporation). We identified tRNA genes with tRNAscan-SE (Lowe and Eddy 1997) or by eye. BLAST searches (Altschul et al. 1997) identified tRNA and protein-coding genes. Hydrophilicity profile comparisons (Hopp and Woods 1981) (MacVector™) were also used to identify or confirm protein-coding genes.

Results

General Features of the mt Genome of T. imaginis

The mt genome of T. imaginis is circular (fig. 1) and has 15,407 bp. The nucleotide composition of the
majority-strand is 6,756 A (44%), 5,041 T (33%), 1,997 C (13%), and 1,613 G (10%). This genome has all the 37 genes commonly found in animal mt genomes. However, unlike most animal mt genomes, this genome has an extra trnS, two pseudo-tRNA genes, and two putative control regions (fig. 1). The majority-strand encodes 31 genes, whereas the minority-strand encodes seven genes. Eight pairs of neighboring genes apparently overlap by 1 to 21 bp: nad5-trnH, trnH-nad4, nad4-nad4L, nad3-trnL2, trnN-trnE, trnA-trnF, atp8-atp6, and trnQ-trnS2 (see Supplementary Material). Four obvious stem-loops were identified: two in the pseudo-tRNA genes and two in the control regions. The gene arrangement in this genome is dramatically different from the putative arrangement of the ancestral hexapods: 24 genes have translocated and eight of these genes have also inverted.

Protein-Coding Genes

BLAST searches failed to identify atp8, nad4L, and nad6; these three genes were identified by comparisons of the hydrophilicity profiles of their putative proteins with those of D. yakuba and Homo sapiens (Anderson et al. 1981; Clary and Wolstenholme 1985) (fig. 2). Among the 3,661 codons of the 13 protein-coding genes, 1,607 (44%) are A+T-rich codons (those with A or T at the first and second codon positions, but termination codons and those coding for leucines were excluded), whereas only 448 (12%) are G+C-rich codons (those with G or C at the first and second codon positions) (Foster, Jermiin, and Hickey 1997) (table 1). Further, 3,186 (87%) codons have A or T at the third codon position, whereas only 475 (13%) codons have G or C at the third codon position (table 2). ATA and ATT apparently initiate the translations of eight and four genes, respectively. Comparisons of hydrophilicity profiles and amino acid sequences of nad4 between T. imaginis and D. yakuba suggest that ATAA probably initiates the translation of nad4 in T. imaginis, as is the case for cox1 of D. melanogaster (de Bruijn 1983), D. yakuba (Clary and Wolstenholme 1985), and Daphnia pulex (Crease 1999). Four genes apparently have incomplete termination codons: TA for nad4 and nad5 and...
Table 2
Nucleotide Composition at Each Codon Position of the Protein-Coding Genes in the Mitochondrial Genome of *Thrips imaginis*

<table>
<thead>
<tr>
<th>Base</th>
<th>1st Position</th>
<th>2nd Position</th>
<th>3rd Position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>%</td>
<td>Count</td>
</tr>
<tr>
<td>A</td>
<td>1306</td>
<td>35.7</td>
<td>743</td>
</tr>
<tr>
<td>T</td>
<td>1304</td>
<td>35.6</td>
<td>1771</td>
</tr>
<tr>
<td>A+T</td>
<td>2610</td>
<td>71.3</td>
<td>2514</td>
</tr>
<tr>
<td>C</td>
<td>468</td>
<td>12.8</td>
<td>678</td>
</tr>
<tr>
<td>G</td>
<td>583</td>
<td>15.9</td>
<td>469</td>
</tr>
<tr>
<td>C+G</td>
<td>1051</td>
<td>28.7</td>
<td>1147</td>
</tr>
</tbody>
</table>

T for *atp8* and *nad2*. TAG apparently terminates the translation of *nad4L*, and TAA terminates the translations of the other eight genes.

tRNA Genes and Pseudo-tRNA Genes

The mt genome of *T. imaginis* has 23 tRNA genes and two pseudo-tRNA genes (fig. 3). Three tRNA genes in *T. imaginis* may code for tRNA-Ser: *trnS1* (ctt), *trnS2* (tga), and *trnS3* (gct). *trnS1* shares more nucleotides with *trnS* (ntc) of other insects than *trnS3* and thus is more likely to be the orthologous copy of *trnS* (ntc) than is *trnS3*. *trnS2* is probably a derived copy of *trnS* (ntc), based on its anticodon sequence and inferred secondary structure. For the mt genomes of invertebrates, all four AGN codons encode serines (Jukes and Osawa 1993). The tRNA-Ser encoded by *trnS2* may be required for the transfer of series encoded by AGC and AGT in *T. imaginis* (table 1); however, this needs to be tested by experiments. Pseudo-*trnE* is identical to a segment of *trnE*, and pseudo-*trnS3* is identical to a segment of *trnS3*. These putative pseudogenes cannot form clover-leaf shaped structures but can form stem-loops.

rRNA Genes

BLAST searches indicate that *rrnS* is between *trnF* and *atp8* and that *rrnL* is between *trnV* and *cox1*. We aligned the nucleotide sequences of these two regions with *rrnS* and *rrnL* of *D. yakuba*, respectively, and estimated the boundaries of *rrnS* and *rrnL* of *T. imaginis*. The arrangement of these two rRNA genes in *T. imaginis* is extraordinary for an arthropod (fig. 1). First, the two rRNA genes are not close to one another; there are 5,705 bp between the 3’ end of *rrnS* and the 5’ end of *rrnL*. In all other arthropods, and in most other animals studied, the two rRNA genes are close to one another (see Boore’s web page). Second, in all other arthropods, the minority-strand encodes the two rRNA genes, whereas in *T. imaginis* the majority-strand encodes these genes. Third, the rRNA genes are adjacent to a control region in all other arthropods and in most animals studied, whereas these genes are distant from either of the putative control regions in *T. imaginis*.

Noncoding Regions

There are apparently 1,136 noncoding nucleotides in the mt genome of *T. imaginis*: 236 bp in 16 intergenic regions and 900 bp in two large noncoding regions. The two large noncoding regions are 440 bp and 460 bp long, and they have 437 bp in common (fig. 4). We propose that these two regions are the control regions since these regions have four features in common with the control regions of other insects (Zhang and Hewitt 1997): (1) tandem repeats, (2) a T-stretch, (3) an A+T-rich (84%) segment, and (4) a stem-loop (figs. 4 and 5).

Arrangement of Genes

Compared with the putative ancestral gene arrangement for hexapods, 24 genes in the mt genome of *T. imaginis* have translocated; eight of these genes have also inverted (fig. 6). These rearrangements comprise translocations of 11 tRNA genes and five protein-coding genes, translocations and inversions of five tRNA genes, two rRNA genes, and one protein-coding gene. Only seven of the 38 ancestral gene boundaries are present in *T. imaginis*: *trnL1-cox2*, *nad2-trnW*, *atp8-atp6*, *nad4L-nad4-trnH*, *trnL2-cox2*, and *trnV-rrnL*. Also, neither of the control regions is in the inferred ancestral position for hexapods.

Discussion

Transcription of rRNA Genes in *T. imaginis*

Most of what we know about the transcription of rRNA genes in animal mt genome is from studies of mammals, especially *Homo sapiens* (Taamman 1999). Most transcription in the mt genome of *H. sapiens* starts at a promoter (TGA1) in the control region and stops in *rrnL2*. This type of transcription ensures that the rRNA genes are expressed at a much higher rate than other mt genes (Gelfand and Attardi 1981; Montoya et al. 1982; Montoya, Gaines, and Attardi 1983).

Remarkably little is known about the transcription of rRNA genes in arthropods. However, in all arthropods studied, except *T. imaginis*, the two rRNA genes are arranged in a similar way as in *H. sapiens*: the two rRNA genes are close to one another and *rrnS* is adjacent to the control region. Therefore, it is likely that the arthropods studied, other than *T. imaginis*, have the same or similar mechanism of rRNA gene transcription as *H. sapiens*. In *T. imaginis*, however, the two rRNA genes have inverted and are distant from one another. Moreover, both rRNA genes are distant from the putative control regions. This raises a question: How are rRNA genes transcribed in *T. imaginis*? It appears that the most efficient transcription of rRNA genes occurs when the promoter and termination element are adjacent to the rRNA genes, as in *H. sapiens*. If this is the case for *T. imaginis*, then there may be two sets of promoter and termination elements for rRNA gene transcription in *T. imaginis*. Further, the promoters would more likely be in the genes upstream of rRNA genes than in the control regions. Arrangements of rRNA genes similar to that of *T. imaginis* also occur in some mollusks and nematodes (see Boore’s web page). Biochemical studies, such as mapping experiments (Montoya et al. 1982) and mutagenesis experiments (Shadel and Clayton 1993), are needed to elucidate the mechanism of rRNA gene transcription in these organisms.
FIG. 3.—Inferred secondary structures of the 23 tRNAs and two pseudo-tRNAs encoded by the mitochondrial genome of *Thrips imaginis*. The tRNAs are labeled with the abbreviations of their corresponding amino acids. Nucleotide sequences are from 5′ to 3′ as indicated for tRNA-Ala. Each arm and loop is illustrated as for tRNA-Ala: AA-arm for amino acid acceptor arm, T-arm for TΨC arm, V-loop for variable loop, AC-arm for anticodon arm, and D-arm for dihydrouridine arm. The D-arm replacement is shown for tRNA-Ser (ucu). Dashes (−) indicate Watson-Crick bonds, and dots (⋅) indicate bonds between T and G. Pseudo-tRNAs are in dash-line boxes, together with their corresponding segments of the genuine tRNAs.
Duplicate Control Regions and Gene Rearrangements

Duplicate control regions have been found in ticks (Black and Roehrdanz 1998; Campbell and Barker 1998, 1999), sea cucumbers (Arndt and Smith 1998), a fish (Lee et al. 2001), parrots (Eberhard, Wright, and Bermingham 2001), and snakes (Kumazawa et al. 1996, 1998). T. imaginis is the first known species of hexapod which has duplicate control regions and, further, the first species of animal which has both duplicate control regions and rearrangements of the majority of mt genes.

Kumazawa et al. (1998) proposed two models for the evolution of duplicate control regions: (1) gene conversion and (2) tandem duplication and deletion. Southern hybridization experiments on snakes supported the tandem duplication and deletion model but did not exclude the possibility of gene conversion (Kumazawa et al. 1998). Tandem duplication and deletion is also the most plausible model for gene translocations in animal mt genomes (Boore 2000). In fact, except for the fish, all the animals which have duplicate control regions have also had translocations of genes. The duplicate control regions and gene translocations of the above animals, except T. imaginis, can be explained by a single tandem duplication followed by deletions of one copy of the duplicate genes, but retention of both the control regions.

Tandem duplication and deletion is also a plausible model for the translocations of genes and the evolution of duplicate control regions in T. imaginis, although the possibility of gene conversion cannot be excluded. Tandem duplication and deletion may have occurred a number of times in the lineage leading to T. imaginis since the number of genes translocated in T. imaginis (24 genes) is much higher than in ticks (two tRNA genes plus a block of 10 genes), sea cucumbers (six tRNA genes), parrots (a block of three genes), and snakes (one tRNA gene). We noticed that in T. imaginis, all the 18 genes between control regions #1 and #2 have translocated and/or inverted, whereas only six of the 20 genes outside this region have translocated and/or inverted (fig. 6). Further, the extra gene, trnS3, and the two pseudo-tRNA genes are either in this section or immediately adjacent to one of the control regions. These observations suggest that duplicate control regions may facilitate the tandem duplication of the genes between them.

Intramitochondrial recombination (Dowton and Austin 1999; Dowton and Campbell 2001) may account for the gene inversions in the mt genome of T. imaginis. All the eight genes which have inverted in T. imaginis are on the minority-strand of the mt genome of the putative ancestral hexapod (fig. 6). Five of these genes, nad1, trnL1, trnL2, trnV and rrnS, probably inverted together since theses genes are in one block in the ancestral arrangement.
Convergence of Two Novel Gene Boundaries

The interordinal phylogeny of the hemipteroid assemblage is still an unresolved trichotomy (Kristensen 1991) (fig. 7a). One of the aims of our study was to see if mt gene rearrangements could shed light on this trichotomy. It is generally accepted that the hemipteroid assemblage, Phthiraptera, Psocodea (Phthiraptera + Psocodea), Thysanoptera, and Hemiptera are monophyletic (Boudreaux 1979, pp. 139–143; Kristensen 1991; Wheeler et al. 2001), whereas the Psocoptera may be paraphyletic (Lyal 1985).

Rearranged mt genomes have not been found in the Hemiptera, but have been found in all species studied from the Phthiraptera, Psocodea, and Thysanoptera (Shao et al. 2001). However, of the hemipteroid insects studied, only Thrips imaginis (Thysanoptera) and a lepidopsocid species (Psocodea: Trogomorpha) (Shao et al. 2001) have the Psocodea may be paraphyletic (Lyal 1985).

Convergence of Two Novel Gene Boundaries

Therefore, if the novel gene boundaries shared by T. imaginis and the lepidopsocid species are synapomorphies, the ancestral arrangements of C. quercus will have to be evolutionary reversals (fig. 7c), which is less likely, or the Psocodea will have to be paraphyletic (fig. 7d), which conflicts with the generally accepted hypothesis that the Psocodea is monophyletic (Boudreaux 1979, pp. 139–143; Kristensen 1991; Wheeler et al. 2001).

Further, we noticed that T. imaginis also shares cob-trnR and another novel gene boundary, cob-trnY, with the hermit crab, Pagurus longicarpus (Crustacea) (Hickerson and Cunningham 2000). It is almost certain that cob-trnR and cob-trnY have evolved independently in T. imaginis and P. longicarpus. Based on the above evidence, we propose that the two novel gene boundaries shared by T. imaginis and the lepidopsocid species evolved by convergence and thus are not informative for the resolution of interordinal phylogeny of the hemipteroid assemblage.

Hemipteroid Assemblage As a Model System

The hemipteroid assemblage is a good model system for studies of the evolution of animal mt genomes for two reasons. First, various gene rearrangements have occurred in the hemipteroid assemblage: translocations and inversions of rRNA genes, tRNA genes, and protein-
These novel gene boundaries are synapomorphies. (by *T. imaginis* (Phthiraptera). (interordinal phylogeny of the hemipteroid assemblage. (*Psocoptera) would then have to be interpreted as evolutionary reversals, or conclude that the novel gene boundaries shared by *T. imaginis* and the lepidopsocid species (*Psocodea*) are sister groups if these novel gene boundaries are synapomorphies. (c) However, the ancestral gene boundaries *cox2-trnK*, *cox3-trnG*, and *trnA-trnR* (A) in *Caecilius quercus* (*Psocodea*) would then have to be interpreted as evolutionary reversals, or (d) the Psocodea would have to be interpreted as paraphyletic. We conclude that the novel gene boundaries shared by *T. imaginis* and the lepidopsocid species evolved by convergence and thus are not informative for the interordinal phylogeny of the hemipteroid assemblage.

Coding genes, evolution of duplicate control regions, and evolution of extra tRNA gene and pseudogenes. Second, there is much variation in the rate of gene rearrangement among the four orders of hemipteroid insects. The number of genes rearranged ranges from zero in *T. dimidiata* (Hemiptera) (Dotson and Beard 2001) to eight in the lepidopsocid species (*Psocodea*) (Shao et al. 2001) to 24 in *T. imaginis* (Thysanoptera) and to 31 in *H. macropus* (Phthiraptera) (Shao, Campbell, and Barker 2001).

Questions which may be addressed with the hemipteroid assemblage include (1) Why does the rate of gene rearrangement vary so much among closely related lineages? (2) What are the mechanisms of gene rearrangement? (3) How are the rRNA genes which are distant from one another and from the control region transcribed? (4) How do the mt genomes which have duplicate control regions replicate? (5) Is the rate of gene rearrangement correlated with the rate of nucleotide substitution? (6) What types of gene rearrangements are more reliable as phylogenetic markers?

**Supplementary Material**

See the accompanying online file for the annotated mtDNA sequence of *T. imaginis*.

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**Literature Cited**


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