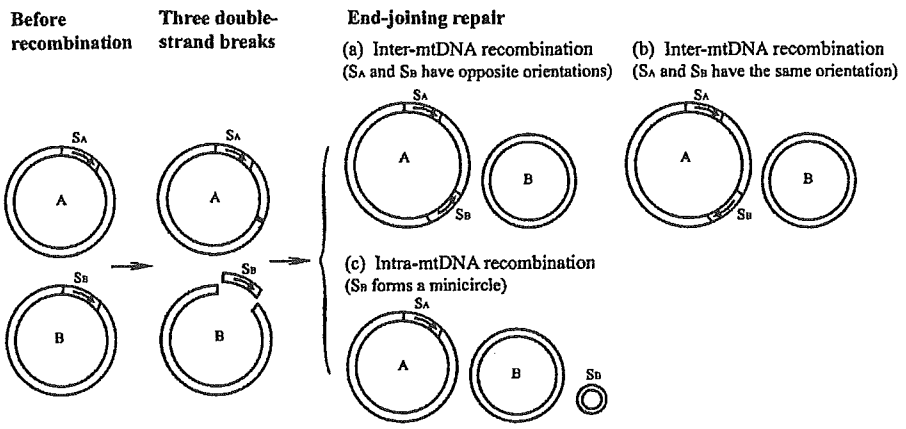

Fig. 4. Model of illegitimate inter-mtDNA recombination. A and B are two identical mt genomes. Consider three double-strand breaks: one break in genome A and two breaks in genome B. A section in genome B, S_B, is cleaved out. If S_B then joins genome A at the break in an orientation opposite to that of its counterpart in genome A, S_A, genome A will have two identical sections, S_A and S_B, which are in opposite orientations (a). This may have occurred in the chigger mite, *Leptotrombidium pallidum*, which has two identical sections, in opposite orientation (see Fig. 1). If S_B joins genome A in the same orientation as that of S_A, genome A will have two identical sections, S_A and S_B, in the same orientation (b). This may have occurred in several animals which have duplicate large noncoding regions (see Shao and Barker 2003). Another way of end-joining, illegitimate intra-mtDNA recombination, will lead to the minicircle S_B (c), which was reported in a nematode (Lunt and Hyman 1997).

uncontrolled cell growth if not properly repaired (Tsukamoto and Ikeda 1998; Pfeiffer et al. 2000).

(2) *End-joining repair*: Double-strand breaks are repaired in two ways in eukaryotes: (1) homologous recombination and (2) illegitimate recombination (non-homologous end-joining; Tsukamoto and Ikeda 1998; Pfeiffer et al. 2000; van Rijk and Bloemendal 2003). Nonhomologous end-joining is apparently the main way that double-strand breaks are repaired in the nuclei of mammalian cells (Tsukamoto and Ikeda 1998). Repair of double-strand breaks in animal mitochondria is still poorly understood. However, it has been shown in mammals that mt protein extracts can catalyze end-joining of linearized DNA molecules. This indicates that end-joining repair of double-strand breaks may also occur in mitochondria (Lakshmipathy and Campbell 1999). If the cleaved section, S_B, joins genome A at the break point in an orientation that is opposite to that of its counterpart in genome A, S_A, genome A will have two identical sections, S_A and S_B, in opposite orientations (Fig. 4a). The mt genome of the chigger mite, *L. pallidum*, may have been formed in this way. There are, however, other possible outcomes of this type of repair. One outcome is S_A and S_B in the same orientation in genome A (Fig. 4b). This type of gene arrangement was found in several animals which have well-separated duplicate LNRs in the same orientations of transcription (Kumazawa et al. 1998; Shao and Barker 2003). However, tandem duplication followed by deletions of genes could also account for this type of gene arrangement (Kumazawa et al. 1998). Another outcome is the two ends of S_B rejoining to form a minicircle (Fig. 4c); this was reported in a nematode (Lunt and Hyman 1997).

Three questions remain unanswered: (1) What is the phylogenetic extent of the novel mt gene content and gene arrangement observed in *L. pallidum*? (2) Is the 16,779-bp mt genome we sequenced with novel gene content and gene arrangement fixed in *L. pallidum*, or is it an intermediate type of mt genome present only in some individuals or populations of *L. pallidum*? and (3) Are the identical nt sequences of the two distantly separated sections (Sections #1 and #2) in the mt genome of *L. pallidum* attributable to concerted evolution or a very recent event of inter-mtDNA recombination? A broad sample of *L. pallidum* from different populations, and their kin, is needed to address these questions.

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Evolution of Duplicate Control Regions in the Mitochondrial Genomes of Metazoa: A Case Study with Australasian *Ixodes* Ticks

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To investigate the evolution pattern and phylogenetic utility of duplicate control regions (CRs) in mitochondrial (mt) genomes, we sequenced the entire mt genomes of three *Ixodes* species and part of the mt genomes of another 11 species. All the species from the Australasian lineage have duplicate CRs, whereas the other species have one CR. Sequence analyses indicate that the two CRs of the Australasian *Ixodes* ticks have evolved in concert in each species. In addition to the Australasian *Ixodes* ticks, species from seven other lineages of metazoa also have mt genomes with duplicate CRs. Accumulated mtDNA sequence data from these metazoans and two recent experiments on replication of mt genomes in human cell lines with duplicate CRs allowed us to re-examine four intriguing questions about the presence of duplicate CRs in the mt genomes of metazoa: (1) Why do some mt genomes, but not others, have duplicate CRs? (2) How did mt genomes with duplicate CRs evolve? (3) How could the nucleotide sequences of duplicate CRs remain identical or very similar over evolutionary time? (4) Are duplicate CRs phylogenetic markers? It appears that mt genomes with duplicate CRs have a selective advantage in replication over mt genomes with one CR. Tandem duplication followed by deletion of genes is the most plausible mechanism for the generation of mt genomes with duplicate CRs. Once duplicate CRs occur in an mt genome, they tend to evolve in concert, probably by gene conversion. However, there are lineages where gene conversion may not always occur, and, thus, the two CRs may evolve independently in these lineages. Duplicate CRs have much potential as phylogenetic markers at low taxonomic levels, such as within genera, within families, or among families, but not at high taxonomic levels, such as among orders.

Introduction

The mitochondrial (mt) genomes of metazoa are typically circular, are about 15 to 20 kb long, and contain 37 genes and a large noncoding region (LNR [Boore 1999]). mt Genomes of metazoa are usually compact: there are no introns and few noncoding nucleotides, except in the LNR (Attardi 1985). For several metazoans, the LNR has been shown to contain elements that control transcription of mt genes and/or replication of mt genomes; this region is, therefore, commonly called the control region (CR). For other metazoans, the largest noncoding region in an mt genome is generally assumed to be the CR (Boore 1999).

CRs may contain the initiation sites of mt gene transcription. Two of the three transcription-initiation sites in the mt genome of *Homo sapiens* are in the CR (Taanman 1999). All of the transcription-initiation sites identified so far in other vertebrates are in the CR (Tracy and Stern 1995). Two of the five initiation sites in the mt genome of *Drosophila melanogaster* are in the CR (Berthier et al. 1986). The sea urchin, *Paracentrotus lividus*, is the only exception known so far: neither of its two known transcription-initiation sites is in the CR (Cantatore et al. 1990).

CRs may also contain the initiation sites for the replication of mt genomes. Two models have been proposed for the replication of mt genomes in mammals: the strand-displacement model (Clayton 1982) and the strand-coupled model (Holt, Lorimer, and Jacobs 2000). According to the strand-displacement model, replication of one strand (the leading strand) initiates at the CR, whereas replication of the other strand (the lagging strand) initiates at a site distant from the CR. According to the strand-coupled model, replications of both strands initiate at the CR. These two models agree that the replication of the leading strand initiates at the CR, although they disagree on the initiation sites of the replication of the lagging strand, and there is also debate over which model predominates in mammalian cells (Bogenhagen and Clayton 2003; Holt and Jacobs 2003). The replication mechanism of mammalian mt genomes is thought to be conserved in vertebrates (Shadel and Clayton 1997) but not conserved in invertebrates (Rubenstein, Brutlag, and Clayton 1977). However, it is known that replications of leading strands in the mt genomes of fruitflies also initiate at the CR (Goddard and Wolstenholme 1980).

The mt genomes of most metazoa studied to date have only one CR. However, the mt genomes of some snakes (Kumazawa et al. 1996), sea cucumbers (Arndt and Smith 1998), metastriate ticks (Black and Roehrdanz 1998; Campbell and Barker 1999), *Amazona* parrots (Eberhard, Wright, and Bermingham 2001), a fish (Lee et al. 2001), a thrips (Shao and Barker 2003), and a sea firefly (Ogoh and Ohmiya 2004) have duplicate CRs; that is, two separate CRs with identical or highly similar nucleotide (nt) sequences. The lineage of snakes has had duplicate CRs for over 70 Myr, whereas the lineage of metastriate ticks has had duplicate CRs for over 210 Myr (Kumazawa et al. 1996; Campbell and Barker 1999). Some humans with mt disorders also have mt genomes with duplicate CRs; these patients usually have a mixture of wild-type mt genomes (one CR), partially deleted mt genomes (one CR), and partially duplicated mt genomes (two CRs) in their clinically affected tissues (Schon, Bonilla, and DiMauro 1997).

The presence of duplicate CRs in the mt genomes of metazoa is an intriguing mutational phenomenon in light

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of the otherwise extreme economy of these genomes. This phenomenon raises at least four functional and evolutionary questions (Kumazawa et al. 1996, 1998; Tang et al. 2000; Umeda et al. 2001). Why do some mt genomes, but not others, have duplicate CRs? How did mt genomes with duplicate CRs evolve? How could the nt sequences of duplicate CRs remain identical or highly similar over evolutionary time? Are duplicate CRs phylogenetic markers? Here, we present analyses of the entire and/or partial mtDNA sequences of 14 species of *Ixodes* ticks. We show that the Australasian *Ixodes* have duplicate CRs that evolved in concert in each species. Further, we address the four questions above with accumulated mtDNA sequences of metazoa with duplicate CRs and two recently published experiments on the replication of mt genomes in human cell lines with duplicate CRs.

Materials and Methods

Sequencing of the mt Genomes of *Ixodes* Ticks

Our methods of DNA extraction, PCR amplification, sequencing, sequence analysis, and annotation of mt genomes are described in Shao et al. (2001, 2004). The primers used in the present study are (1) nad1F (5′-TTTTATTTGGCCCTTTTCGAA-3′), (2) rrnLR1 (5′-CTGCTCAATGATTTTTTAAATTGCTGTGG-3′), (3) rrnLR2 (5′-WWGWTTGCGACCTCGATGTTG-3′), (4) rrnLR3 (5′-AAGTTACCTTAGGGATAACAGCGT-3′); (5) rrnSF1 (5′-GGCGATATGTGCATATTCTAGAGC-3′), (6) rrnSF2 (5′-GTATRACCGCGRWTGCTGGCAC-3′), (7) rrnSF3 (5′-AATAATAGGGTATCTAATCC-3′), (8) rrnSF4 (5′-TACTATGTTACGACTTAT-3′), (9) trnMR1 (5′-TGGGGTATGAACCCAGTAGC-3′), (10) trnMR2 (5′-TTTGGGGTATGAACCCACTAGC-3′), (11) trnL2F1 (5′-TTCCTCGCATTATATCTGCCACTT-3′), (12) trnL2F2 (5′-TCATAAAGGAAGCTTAAATTCC-3′), and (13) trnQR (5′-GCACGATAAATTTTGATTTTA-3′). The combinations of primers used for each species are shown in table 1.

Phylogenetic Analyses of the nt Sequences of CRs

The sequences of the CR of *Ixodes* ticks determined in this study and the sequences of the CR of other ticks in GenBank were aligned with ClustalX (Thompson et al. 1997). For both pairwise and multiple alignments, the gap opening penalty was 15.00 and the gap extension penalty was 6.66. For multiple alignment, the delay divergent sequence was 30% and the DNA transition weight was 0.50. Neighbor-Joining (NJ), maximum-likelihood (ML), and maximum-parsimony (MP) trees were constructed with PAUP* version 4.0 (Swofford 2000). The general time reversible model and gamma distributed rates (Lanave et al. 1984) were used in ML tree construction; the instantaneous rate matrix, base frequencies, and the shape of gamma distribution were estimated by PAUP*. Bootstrap tests (1,000 replicates) were run on the NJ and the consensus MP trees. The horseshoe crab, *Limulus polyphemus* (Lavrov, Boore, and Brown 2000), was used for outgroup reference.

Results

Mitochondrial Genomes of *Ixodes* Ticks

Duplicate CRs were originally noticed in partial sequences of mt genomes of *I. holocyclus* and *I. uriae* in a phylogenetic study. To check that these CRs were not artifacts or pseudo mt genes from nuclear genomes (Lopez et al. 1994), the entire mt genomes of *I. persulcatus*, *I. holocyclus*, and *I. uriae* were amplified by PCR and sequenced. These three genomes are circular and 15,007 bp, 15,053 bp, and 14,539 bp long, respectively; all three genomes have the 37 genes and other features typical of the mt genomes of metazoa. The arrangement of 37 mt genes in these three *Ixodes* species is the same as that of the hypothetical ancestor of the arthropods (Lavrov, Boore, and Brown 2000 [fig. 1]). The mt genome of *I. persulcatus* has one CR, between the small rRNA gene (*rrnS*) and the tRNA-Ile gene (*trnI*); this position is the ancestral position of the CR for arthropods. However, *I. holocyclus* and *I. uriae* have two CRs of similar size and highly similar nt sequence: CR#1 is in the ancestral position for arthropods, whereas CR#2 is between tRNA-Leu gene ($trnL_1$; anticodon tag) and large rRNA gene (*rrnL*), which is novel to arthropods (fig. 1).

The genus *Ixodes* has 249 species and is the largest genus of the family Ixodidae (hard ticks, 713 species) and the suborder Ixodida (ticks, 899 species [Barker and Murrell 2004]). To examine the evolution pattern and the phylogenetic utility of the duplicate CRs found in *I. holocyclus* and *I. uriae*, the entire CR and part of the flanking genes of another 11 species of *Ixodes* were sequenced. The presence/absence of duplicate CRs in another four *Ixodes* species was tested by PCR. Sequencing and PCR test revealed that duplicate CRs were also present in another six species of *Ixodes*: *I. antechini*, *I. cordifer*, *I. cornuatus*, *I. hirsti*, *I. myrmecobii*, and *I. trichosuri* but were not present in *I. acutitarsus*, *I. asanumai*, *I. loricatus*, *I. ovatus*, *I. pilosus*, *I. ricinus*, *I. scapularis*, *I. simplex*, and *I. turdus* (table 1). Further, CR#1 of *I. myrmecobii* has two tandem repeats, CR#1a and CR#1b. All eight species of *Ixodes* ticks that have two CRs are from the Australasian lineage of *Ixodes* (*sensu* Barker and Murrell [2004]). These ticks live only in Australasia; that is, Australia, New Guinea, New Zealand, and some islands of the Pacific and Indian oceans, except for *I. uriae*, which infests sea birds that live both within and without Australasia. These ticks are referred to as the Australasian *Ixodes* hereafter. The nt sequences of the *Ixodes* ticks determined in this study were deposited in GenBank under the accession numbers shown in table 1.

Duplicate CRs of Australasian *Ixodes* Ticks

The two CRs of the seven Australasian *Ixodes* species sequenced in this study were 349 to 476 bp long (table 2). The nt sequences of CR#1 and CR#2 of a species were 87% to 95% similar. The nt sequences of CR#1 and CR#2 of a species were more similar to each other than were the CR#1 sequences or the CR#2 sequences of different species. Consider *I. cornuatus* and *I. myrmecobii*, which are apparently sister species (fig. 2). CR#1 and CR#2 of

Table 1
Species of Ticks Studied

Species	Location of Collection	Collector or Reference	Number of CRs	Primers for CR#1[a]	Primers for CR#2[b]	Accession Numbers
Australasian *Ixodes* ticks						
I. antechini (P)	Queensland, Australia	Matthew Shaw (B1569)	2	rrnSF2 and trnMR1	nad1F and rrnLR3	PCR test
I. cordifer (P)	Queensland, Australia	David Spratt (B2046)	2	rrnSF3 and trnMR1	trnL2F2 and rrnLR3	AB161424-5*
I. cornuatus (P)	Tasmania, Australia	Peter McKean (B906)	2	rrnSF3 and trnMR1	trnL2F2 and rrnLR3	AB161426-7*
I. hirsti (P)	Victoria, Australia	David Kemp (B1579)	2	rrnSF3 and trnMR1	trnL2F2 and rrnLR3	AB161428-9*
I. holocyclus (C)	Sydney, Australia	Minoru Nakao and Masahito Fukunaga	2	NA	NA	AB075955*
I. myrmecobii (P)	Western Australia	Matthew Shaw (B1546)	2	rrnSF4 and trnMR1	nad1F and rrnLR2	AB161432-3*
I. trichosuri (P)	ACT, Australia	David Spratt (B2033)	2	rrnSF3 and trnMR1	trnL2F2 and rrnLR2	AB161442-3*
I. uriae (C)	Bonden, Sweden	Asa Gylfe and Sven Bergstrom	2	NA	NA	AB087746*
Other *Ixodes* ticks						
I. acutitarsus (P)	Aomori, Japan	Hiromi Fujita	1	rrnSF1 and trnQR	trnL2F1 and rrnLR1	AB161176*
I. asanumai (P)	Lab strain, Tokyo, Japan	Fumio Hayashi	1	rrnSF1 and trnQR	trnL2F1 and rrnLR1	PCR test
I. hexagonus (C)	NA	Black and Roehrdanz 1998	1	NA	NA	AF081828
I. loricatus (P)	Sao Paulo, Brazil	Marcelo Labruna (B2079)	1	rrnSF3 and trnMR2	trnL2F2 and rrnLR3	AB161430-1*
I. ovatus (P)	Hokkaido, Japan	Minoru Nakao	1	rrnSF1 and trnQR	trnL2F1 and rrnLR1	PCR test
I. persulcatus (C)	Hokkaido, Japan	Minoru Nakao	1	NA	NA	AB073725*
I. pilosus (P)	South Africa	I. Mckay (B359)	1	rrnSF2 and trnMR2	nad1F and rrnLR3	AB161434-5*
I. ricinus (P)	Slovak Republic	J. Rehacek (B774)	1	rrnSF2 and trnMR2	nad1F and rrnLR3	AB161436-7*
I. scapularis (P)	Lab strain, CDC, USA	Joseph Piesman (B2078)	1	rrnSF3 and trnMR2	trnL2F2 and rrnLR3	AB161438-9*
I. simplex (P)	Queensland, Australia	Martin Schulz (B538)	1	rrnSF2 and trnMR2	trnL2F2 and rrnLR3	AB161440-1*
I. turdus (P)	Saitama, Japan	Kazuyoshi Fujimoto	1	rrnSF1 and trnQR	trnL2F1 and rrnLR1	PCR test
Metastriate ticks						
Amblyomma triguttatum (C)	Queensland, Australia	Shao et al., unpublished (B1545)	2	NA	NA	AB113317
A. vikirri (P)	NA	Murrell, Campbell, and Barker 2003	2	NA	NA	AY059179
A. varanensis (P)	NA	Black and Roehrdanz 1998	2	NA	NA	NA
Haemaphysalis flava (C)	NA	Shao et al. 2004	2	NA	NA	AB075954
Hyalomma truncatum (P)	NA	Murrell, Campbell, and Barker 2003	2	NA	NA	AY059271-2
Rhipicephalus appendiculatus (P)	NA	Murrell, Campbell, and Barker 2003	2	NA	NA	AY059216-7
R. (Boophilus) microplus (*P*)[c]	NA	Campbell and Barker 1999	2	NA	NA	AH007623
R. sanguineus (C)	NA	Black and Roehrdanz 1998	2	NA	NA	AF081829
Soft ticks						
Carios capensis (C)	NA	Shao et al. 2004	1	NA	NA	AB075953
Ornithodoros moubata (C)	NA	Shao et al. 2004	1	NA	NA	AB073679
O. porcinus (C)	Dodoma, Tanzania	Fukunaga, unpublished.	1	NA	NA	AB105451
Horseshoe crab						
Limulus polyphemus (C)	NA	Lavrov, Boore, and Brown 2000	1	NA	NA	NC_003057

NOTE.—CR indicates control region. C indicates complete mtDNA. P indicates partial mtDNA. Asterisks indicate sequences generated in this study.
[a] Between *rrnS* and *trnI*.
[b] Between $trnL_I$ and *rrnL*.
[c] *Rhipicephalus* (*Boophilus*) *microplus* was formerly *Boophilus microplus* (Barker and Murrell 2004).

I. cornuatus were 95% similar, and CR#1 and CR#2 of *I. myrmecobii* were 90% similar. However, CR#1 of *I. cornuatus* and CR#1 of *I. myrmecobii* were only 81% similar, and CR#2 of *I. cornuatus* and CR#2 of *I. myrmecobii* were only 78% similar. Seven motifs, 5 to 18 nts long, respectively, were conserved among CR#1 and CR#2 of the seven Australasian *Ixodes* species we sequenced (fig. 3); these motifs were also partially conserved in the other *Ixodes* species, which have one CR (data not shown). The conservation of these motifs

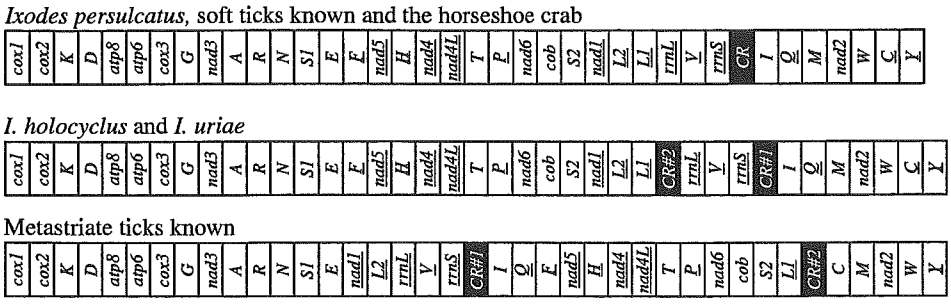


FIG. 1.—The mitochondrial genomes of *Ixodes persulcatus*, *I. holocyclus*, *I. uriae*, and metastriate ticks known. For the purpose of presentation, the circular mt genomes are linearized at the boundary between *cox1* and Y. Genes and control regions are shown as boxes. Control regions are abbreviated as CR and are highlighted in black. The names of genes are in italics: *atp6* and *atp8* for ATP synthase subunits 6 and 8, *cox1-3* for cytochrome oxidase subunits 1 to 3, *cob* for cytochrome *b*, *nad1-6* and *nad4L* for NADH dehydrogenase subunits 1 to 6 and 4L, and *rrnL* and *rrnS* for large and small rRNA subunits. tRNA genes are named with their single-letter amino acid abbreviations. The two genes for tRNA leucine and tRNA serine are differentiated by L_1 (anticodon tag), L_2 (taa), S_1 (tct), and S_2 (tga). Genes are transcribed from left to right, except those genes whose names are underlined; underline genes are transcribed from right to left.

indicates that they may have roles in the control of replication and/or transcription of the mt genome of these ticks.

Phylogenetic Trees of CR Sequences of Ticks

Our phylogenetic analyses of the CR sequences of ticks produced an NJ tree, a ML tree, 47 equally parsimonious MP trees, and a consensus 50% majority-rule MP tree. These trees were largely congruent with each other; therefore, only the consensus MP tree was shown (fig. 2). The NJ, ML, and MP trees were consistent in two groupings. First, the duplicate CRs of a species of Australasian *Ixodes* tick or metastriate tick were always grouped together. Second, the species of ticks were always grouped into four major lineages: the Australasian *Ixodes* ticks, the other *Ixodes* ticks, the soft ticks, and the metastriate ticks. These groupings concur with the current working hypothesis of the phylogeny of the subfamilies and families of ticks proposed from analyses of phenotypes, three nuclear genes (SSU rRNA, LSU rRNA, and ITS2 rRNA), and three mt genes (*rrnS*, *rrnL*, and *cox1* [Barker and Murrell 2004]). The differences among the NJ, ML, and MP trees were in the grouping of the species within the lineages of Australasian *Ixodes* ticks, other *Ixodes* ticks, and metastriate ticks, which were shown as polytomies in the consensus MP tree (fig. 2).

Species of the four major lineages of ticks in our CR-sequence tree differ in the copy number of CR and the arrangement of genes in mt genomes (figs. 1 and 2). The most parsimonious explanation for the evolution of the copy number of CR and the arrangement of mt genes in ticks is that (1) the most recent common ancestor of ticks had one CR and the arrangement of mt genes of the hypothetical ancestor of the arthropods, which has persisted in the horseshoe crab, *Limulus polyphemus* (Lavrov, Boore, and Brown 2000 [fig. 2]); (2) the CR duplicated in the lineage of the Australasian *Ixodes* ticks but no genes rearranged; (3) the CR duplicated and several genes rearranged in the lineage of the metastriate ticks; and (4) a single CR and the ancestral arrangement of genes of ticks persisted unchanged in the other *Ixodes* species and the soft ticks.

Discussion

Concerted Evolution of Duplicate CRs of Australasian *Ixodes* Ticks

Three lines of evidence indicate that the two CRs have evolved in concert in each species of the Australasian *Ixodes*: (1) the nt sequences of CR#1 and CR#2 of a species are highly similar; (2) the nt sequences of CR#1 and CR#2 of the same species are more similar to one another than either is to its namesake in other species; for example, CR#1 and CR#2 of *I. cordifer* are more similar to one another than are CR#1 of *I. cordifer* and CR#1 of any other species of tick (data not shown but see figure 3); and (3) CR#1 and CR#2 of a species were always clustered together in either the NJ, ML, or MP trees.

In addition to the Australasian *Ixodes* ticks and the metastriate ticks, duplicate CRs have also been found in the mt genomes of some snakes (Kumazawa et al. 1996), sea cucumbers (Arndt and Smith 1998), *Amazona* parrots (Eberhard, Wright, and Bermingham 2001), a fish (Lee et al. 2001), a plague thrips (Shao and Barker 2003), and a sea firefly (Ogoh and Ohmiya 2004 [table 2]). Once duplicate CRs occur in an mt genome, they may evolve either in concert or independently. Independent evolution leads to the divergence of the nt sequences of the two CRs and, eventually, degeneration or deletion of one of the CRs (Bensch and Harlid 2000). Concerted evolution, however, keeps the nt sequences of the two CRs highly similar. Kumazawa et al. (1996) and Arndt and Smith (1998) proposed concerted evolution as an explanation for the high similarity of the nt sequences of the two CRs in the snakes and the sea cucumbers they studied. Black and Roehrdanz (1998) and Eberhard, Wright, and Bermingham (2001) showed that the two CRs evolved in concert in each species of the metastriate ticks and the *Amazona* parrots. Black and Roehrdanz (1998) and Eberhard, Wright, and Bermingham (2001) studied two and four species, respectively. In the present study, we studied 26 species of ticks: 15 had duplicate CRs, and 11 had one CR. Our study, together with previous studies, shows conclusively that duplicate CRs in the mt genomes of metazoa tend to evolve in concert in each species rather than independently.

Table 2
Species of Metazoa with Duplicate Control Regions in Their Mitochondrial Genomes

Species	CR#1[a] (bp)	CR#2 (bp)	Similarity Between CR#1 and CR#2[b]
Australasian *Ixodes* ticks			
Ixodes cordifer	364	390	89%
I. cornuatus	357	403	95%
I. hirsti	361	436	93%
I. holocyclus	352	450	87%
I. myrmecobii	353/352[c]	398	90%
I. trichosuri	349	425	94%
I. uriae	388	476	91%
Metastriate ticks			
Amblyomma triguttatum	307	307	93%
A. vikirri	306	312	92%
A. varanensis	309	271	98%
Haemaphysalis flava	310	310	96%
Hyalomma truncatum	312	308	93%
Rhipicephalus appendiculatus	306	303	96%
R. (Boophilus) microplus	302	299	98%
R. sanguineus	305	303	93%
Snakes			
Dinodon semicarinatus	1018	1018	100%
Crotalus viridis	1020	1025	100%
Ovophis okinavensis	1033	1033	100%
Sea cucumbers			
Cucumaria miniata	459	410	97%
Parrots[d]			
Amazona farinose	1553	1457	94%
A. ochrocephala oratrix	1551	1838	92%
A. o. auropalliata	1705	1867	87%
Fish			
Rivulus marmoratus	887	795	95%
Plague thrips			
Thrips imaginis[e]	440	460	99%
Sea firefly			
Vargula hilgendorfii[e]	771	771	99%

[a] CR#1 is at the ancestral position; CR#2 is at a novel position.
[b] Similarity between CR#1 and CR#2 = (number of shared nt/length of alignable nt sequence) × 100%.
[c] Length of CR#1a and CR#1b, respectively.
[d] Both CRs may be in the ancestral positions for birds (see *Discussion*).
[e] Both CRs are in novel positions for arthropods.

The presence of duplicate CRs in the mt genome of metazoa is an intriguing mutational phenomenon in light of the otherwise extreme economy of these genomes. This phenomenon raises several functional and evolutionary questions.

Why Do Some mt Genomes, but Not Others, Have Duplicate CRs?

Replication of the mt genomes of the mammals and fruitflies studied initiates at the CR (Goddard and Wolstenholme 1980; Clayton 1982; Holt, Lorimer, and Jacobs 2000). So, it is probably reasonable to speculate that mt genomes with duplicate CRs may have a selective advantage over mt genomes with one CR. For example, an mt genome with two CRs may replicate more efficiently than an mt genome with one CR (Kumazawa et al. 1996; Arndt and Smith 1998; Umeda et al. 2001). Initiation of replication is apparently a rate-limiting step in the replication of the mt genome of *D. melanogastor* (Rubenstein, Brutlag, and Clayton 1977). If replication can initiate at both CRs, then mt genomes with two CRs could start more replication per unit time than could mt genomes with one CR. Thus, mt genomes with two CRs may "out compete" mt genomes with one CR.

Our knowledge of the replication of metazoan mt genomes is almost entirely from studies of mammals and fruitflies that have one CR. It is not clear yet how mt genomes with two CRs replicate. Nevertheless, two recent experimental studies indicate that an mt genome with two CRs may replicate more efficiently than an mt genome with one CR. Tang et al. (2000) found that the population of mt genomes in human cell lines, which was originally a mixture of genomes with one CR and genomes with two CRs, shifted over time, towards mt genomes with two CRs. This finding suggests that cells may favor mt genomes with two CRs. Tang et al. (2000) proposed that mt genomes with duplicate CRs would have a selective advantage over those with one CR if the two types of genomes were competing for a finite amount of replication factor(s). Further, Umeda et al. (2001) showed that in human cell lines that had partially duplicated mt genomes, the two CRs were equally efficient at starting replication. It is not known, however, whether the two CRs in an mt genome can be active simultaneously, and if so, whether the two CRs start replication simultaneously or sequentially.

How Did mt Genomes with Duplicate CRs Evolve?

Three mechanisms may account for mt genomes with duplicate CRs: tandem duplication, dimerization, and illegitimate recombination (reviewed in Boore [2000]). The tandem duplication mechanism starts with replication errors, such as imprecise termination and/or slipped-strand mispairing. If these errors occur in the section that has the CR, then the replication will generate an mt genome with two tandem-repeated sections, and each section contains a CR. Dimerization occurs when two linearized monomeric mt genomes join "head-to-tail" to form a large circular mt genome. A dimeric mt genome would have two CRs and two copies of each gene. Illegitimate recombination occurs when a section of one mt genome is cleaved out and then introduced into another mt genome. If this section contains the CR, then the mt genome that receives the cleaved section of genome will have two CRs. Obviously, illegitimate recombination may also cause tandem duplications if the introduced section is next to its counterpart in the receipt mt genome.

A single event of tandem duplication followed by deletions of redundant copies of genes can account for the duplicate CRs and/or gene rearrangements in the mt genomes of all of the metazoa known that have duplicate CRs, except the plague thrips and the sea firefly (see Supplementary Material online). However, different genes would have been duplicated and deleted in each of these metazoan lineages. Moreover, the two CRs are in different positions in each lineage. The mt genomes of the plague thrips and the sea firefly are highly rearranged, and,

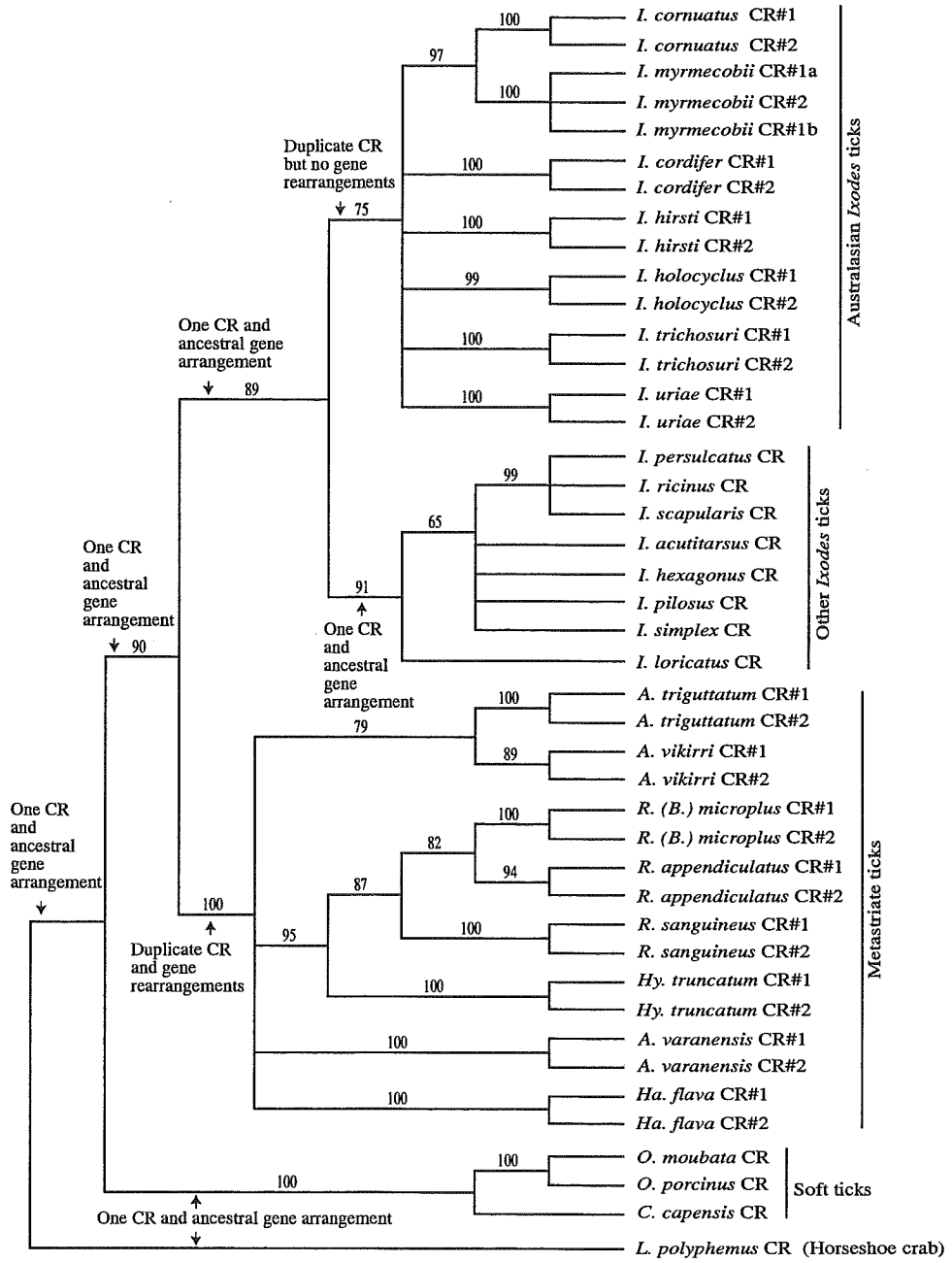


FIG. 2.—The consensus 50% majority-rule maximum-parsimony tree from the nucleotide sequences of the control regions of ticks. Bootstrap support (%; 1,000 replicates) is shown above branches. The horseshoe crab, *Limulus polyphemus*, was the outgroup. See table 1 for the full name of each species.

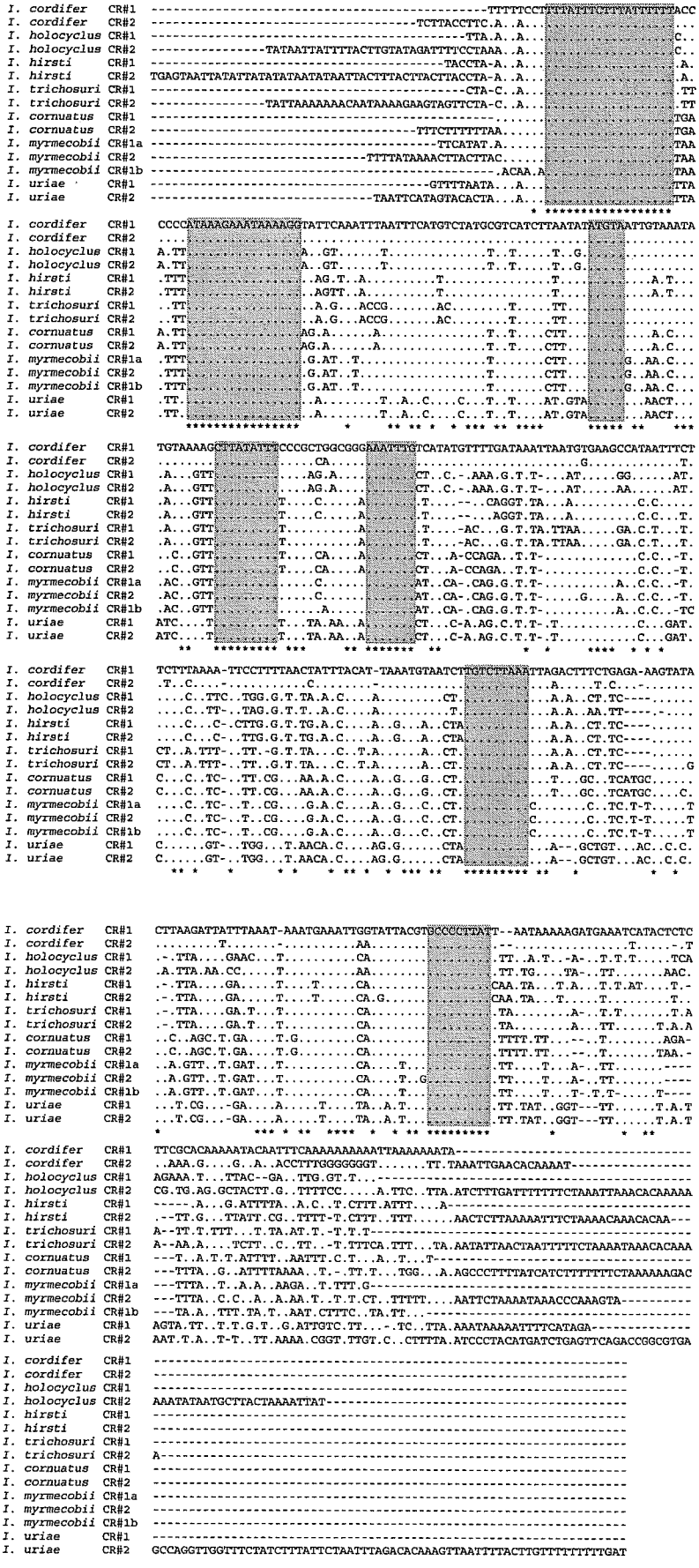
therefore, multiple events of tandem duplication and deletions, and/or illegitimate recombination, may have occurred in the evolution of these genomes (Shao and Barker 2003; Ogoh and Ohmiya 2004).

How Do Duplicate CRs Evolve in Concert?

Kumazawa et al. (1998) proposed two mechanisms for the concerted evolution of duplicate CRs: tandem duplication and gene conversion. The tandem duplication

→

FIG. 3.—Alignment of the nucleotide sequences of the duplicate control regions of seven species of Australasian *Ixodes* ticks. CR#1 is the control region between *rrnS* and *trnI*, and CR#2 is the control region between $trnL_I$ and *rrnL* (see figure 1). There are two copies of CR#1, in tandem, in *I. myrmecobii*: CR#1a is next to *rrnS*, and CR#1b is next to *trnI*. Dashes indicate alignment gaps. Dots indicate nucleotides that are the same as that of CR#1 of *I. cordifer*. Asterisks indicate nucleotides that are conserved among the Australasian *Ixodes* species. Gray-shaded blocks indicate conserved motifs of 5 or more nucleotides.



mechanism starts with a replication error. Replication of a strand that starts in one CR, say CR#1, pauses at the other CR, CR#2. Then the newly synthesized fragment is unwound from the template strand. The two ends of this fragment reanneal to CR#1, and the rest of the fragment forms a loop. Replication of this strand then restarts, and a new strand with three CRs (CR#1, CR#2, and CR#1 with CR#2 in the loop) is synthesized. If the loop is deleted, then replication of the next strand will generate an mt genome with two identical or nearly identical CRs. The gene conversion mechanism involves homologous recombination. The crossing over of nicked strands between the two CRs of an mt genome forms a Holliday structure, which leads to two heteroduplex intermediate CRs. Subsequent DNA repairs replace the nt sequence of one CR with that of the other and lead to two identical CRs in an mt genome.

The tandem duplication mechanism is a less plausible explanation, in our view, for the concerted evolution of duplicate CRs than is the gene conversion mechanism. For the tandem duplication mechanism to account for the concerted evolution of duplicate CRs in each species, the same replication errors must occur over and over again, and independently in each species. This circumstance is less likely. Further, as discussed above, each tandem duplication event could potentially lead to rearrangement of mt genes in a species. However, we did not find differences in mt gene arrangement among the seven species of the Australasian *Ixodes* ticks (two species sequenced entirely; five species sequenced partially), among the eight species of the metastriate ticks (four species sequenced entirely and others sequenced partially), or among three species of the *Amazona* parrots (all sequenced partially [table 2]). Rather, gene conversion is a more plausible mechanism for the concerted evolution of duplicate CRs. Indeed, recombination (homologous and/or nonhomologous) may be an indispensable part of the mtDNA replication and repair machinery of metazoa (Rokas, Ladoukakis, and Zouros 2003). Further, gene conversion can account for the high similarity of the nt sequences of the CRs in each species and the conservation of the gene arrangement among species of the Australasian *Ixodes* ticks, the metastriate ticks, and the *Amazona* parrots, respectively.

Are Duplicate CRs Phylogenetic Markers?

Mitochondrial genomes with duplicate CRs may be a synapomorphy for the Australasian *Ixodes* ticks (fig. 2). Further, duplicate CRs, together with gene rearrangements, is probably a synapomorphy for the metastriate ticks. Kumazawa et al. (1996) suggested that duplicate CRs might be a synapomorphy for the snakes they studied from three genera of two families. Taken together, these studies indicate that duplicate CRs are informative phylogenetic markers at low taxonomic levels such as within a genus, within a family, or among families.

Intriguingly, Mindell, Sorenson, and Dimcheff (1998) and Bensch and Harlid (2000) found that birds from four orders had a novel mt gene arrangement. This novel gene arrangement had a control region (CR) plus a degenerate control region (NC), whereas the typical mt genome of birds has only one CR at the position of NC. These authors showed that the novel gene arrangement evolved by convergence in these lineages of birds and, therefore, was not a synapomorphy. The *Amazona* parrots also had a novel gene arrangement (Eberhard, Wright, and Bermingham 2001). However, instead of a CR and an NC, the *Amazona* parrots had two CRs, CR#1 and CR#2, in the positions of CR and NC in the mt genomes of the four orders of birds studied by Mindell, Sorenson, and Dimcheff (1998) and Bensch and Harlid (2000). The two CRs share high similarity of nt sequences and have evolved in concert (Eberhard, Wright, and Bermingham 2001).

The novel gene arrangement of birds with a CR and an NC was thought to have evolved independently at least five times in the four orders of birds by tandem duplications followed by deletions of genes (Mindell, Sorenson, and Dimcheff 1998; Bensch and Harlid 2000). As discussed above, tandem duplication is a mechanism that involves replication errors. If the novel gene arrangement in birds evolved five times independently, then the same replication errors, same tandem duplications and, subsequently, deletions of the same genes, would have to occur five times independently in the four orders of birds. This circumstance is unlikely, in our view.

Boore (2000) proposed a much more parsimonious explanation for the evolution of the novel mt gene arrangement in the four orders of birds. A gene arrangement with duplicate CRs, CR#1 and CR#2, such as that of the *Amazona* parrots, may be ancestral for all birds. The two CRs may have evolved in concert in some lineages of birds, whereas in other lineages, they may have evolved independently. In the lineages of birds that have two CRs that have evolved independently, one of the two CRs would eventually be deleted or would degenerate; this would lead to either the typical or the novel gene arrangement of birds observed by Mindell, Sorenson, and Dimcheff (1998) and Bensch and Harlid (2000). The apparent convergent evolution in birds of genomes with a CR and an NC suggests that duplicate CRs may not be a reliable phylogenetic marker at high taxonomic levels, such as among orders.

Conclusion

The Australasian *Ixodes* ticks we studied have duplicate CRs that have evolved in concert in each species. To date, mt genomes with duplicate CRs have been found in eight lineages of metazoa. Our analyses of the accumulated mtDNA sequences of metazoans with duplicate CRs and two recent experiments on replication of mt genomes in human cell lines with duplicate CRs indicate that mt genomes with duplicate CRs may have a selective advantage in replication over mt genomes with one control region. Tandem duplication followed by deletion of redundant copies of genes is the most plausible mechanism for the generation of duplicate CRs in mt genomes. Once duplicate CRs occur in an mt genome, they tend to evolve in concert rather than independently, probably by gene conversion. However, in some lineages of metazoa, it seems that gene conversion may not always occur over evolutionary time and, therefore, the two CRs

may evolve independently. Duplicate CRs may be useful phylogenetic markers at low taxonomic levels, such as within a genus, within a family, or among families, but not at high levels, such as among orders of metazoa.

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特集 ウイルス性出血熱

2．ハンタウイルス感染症

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ハンタウイルス感染症はハンタウイルスを病原ウイルスとするヒトの感染症で，げっ歯類によって媒介される重篤な人獣共通感染症である。本症には腎症候性出血熱（hemorrhagic fever with renal syndrome：HFRS）とハンタウイルス肺症候群（hantavirus pulmonary syndrome：HPS）の２つの病型があり，それぞれ腎臓と肺の障害が特徴的である。HFRSの病原ウイルスは東アジア，ヨーロッパ，ロシア，およびわが国などに存在し，HPSの病原ウイルスは南北アメリカ大陸に分布している。病原性のある様々なウイルス型が，それぞれ特有のげっ歯類に保有されて自然界で維持され，今でも新型のハンタウイルスが次々と発見されていることから，ハンタウイルス感染症の予防には流行地と病原巣動物の特定が重要である。

Key Words：ハンタウイルス／人獣共通感染症／げっ歯類

Hantavirus infections

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Hantavirus infections are severe zoonotic diseases in humans which are transmitted by rodents. There are two different types of infections ; one is hemorrhagic fever with renal syndrome (HFRS) and the other is hantavirus pulmonary syndrome(HPS). HFRS and HPS are characterized by renal and pulmonary disorders, respectively. The viruses causing HFRS are distributed in East Asia, Europe, Russia, and Japan while the viruses causing HPS exist in the North and South American Continents. Since variety of hantaviruses pathogenic to humans are maintained in the specific rodents in nature and new hantaviruses have been found year by year, identification of enzootic areas and reservoir animals is important to prevent hantavirus infections.

Key Words：*Hantaviurs ／ Zoonosis ／ Rodent*

はじめに

ハンタウイルスはげっ歯類を病原巣動物として自然界で維持され，世界各国に分布している。本ウイルスがヒトに感染すると腎症候性出血熱 hemorrhagic fever with renal syndrome（HFRS）やハンタウイルス肺症候群 hantavirus pulmonary syndorome（HPS）などの重篤な感染症を引き起こすため，公衆衛生上非常に重要なウイルス性人獣共通感染症の病原体である。ハンタウイルス感染症は腎臓障害や出血などを主徴とするHFRSと急性の呼吸器障害を主徴とするHPSがこれまでに知られている。本稿ではこれら2つのハンタウイルス感染症について概説する。

1930年以降，ユーラシア大陸の各地で発熱や出血傾向とともに腎臓の機能障害(蛋白尿)を特徴とする風土病の存在することが報告されていた[1]。これらの風土病は流行地ごとに中国では流行性出血熱，韓国では韓国型出血熱，さらに北欧では流行性腎症などと呼ばれていた。1978年，韓国の李は北緯38度線近くを流れるハンターン川の河畔で捕獲されたセスジネズミ(図1)から韓国型出血熱の原因ウイルスを分離することに初めて成功し，ハンターンウイルスと命名した[2]。本ウイルスの分離と血清学的な診断法の開発によって，これまで異なった名称で呼ばれていた上記疾患は，いずれも近縁のウイルスによって引き起こされることが判明した。そこで，WHOはこれらの疾患を腎症候性出血熱と統一して呼称することを提案した[3]。これ以後，ハンターンウイルスと抗原的に関連性のあるウイルスをハンタウイルスと総称されることとなった。

これまでに南北アメリカ大陸ではHFRSの発生は報告されていないが，ハンタウイルスに起因する重篤な疾患の存在することが1993年に判明した[4]。1993年5月からアメリカ合衆国の南西部諸州で原因不明の急性の重症型呼吸器疾患が多発し，死亡者が続発した。米国の疾病予防制圧センター（CDC）を中心としたチームが病原体の検索にあたり，本症がシカシロアシマウスが保有するハンタウイルスの感染によって起こることが判明した。本症は肺の機能障害が特徴的なことからハンタウイルス肺症候群と命名された。

病因

HFRSとHPSの病原体はハンタウイルスである。本ウイルスは遺伝子の性状や形態などからブニヤウイルス科の中のハンタウイルス属に分類されるRNAウイルスである[5,6]。ウイルス粒子は直径約100 nmの球形で(図2)，糖蛋白を格子状に配したエンベロープがマイナス鎖で3本の分節状RNAを包んでいる。RNAは分子量の大きい方からL，M，S遺伝子と呼び，それぞれがRNAポリメラーゼ，エンベロープ蛋白質，核蛋白質をコードしている。

ハンタウイルスはこれまで少なくとも20の血清型

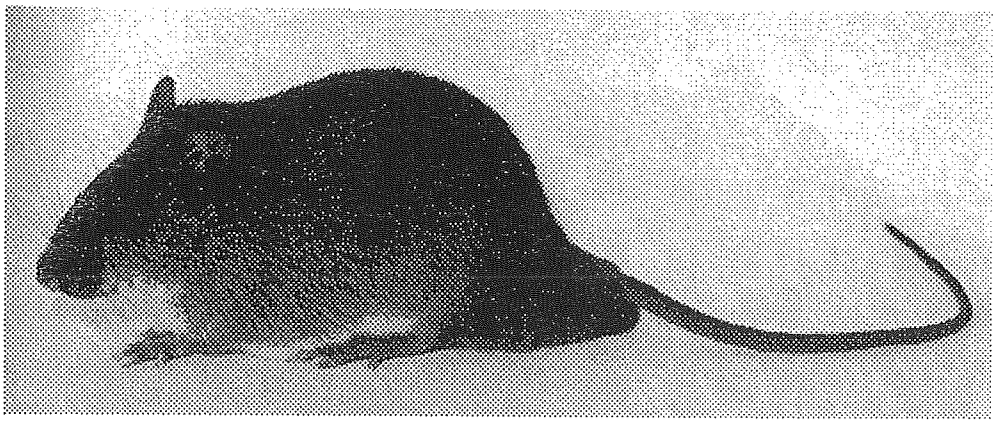

図1　ハンターンウイルスの宿主であるセスジネズミ

ユーラシア大陸東部に生息するセスジネズミはハンターンウイルスを媒介する。極東ロシア，中国，および韓国などで重症型腎症候性出血熱（HFRS）の病原巣動物となっている。

（写真提供：土屋公幸 博士）

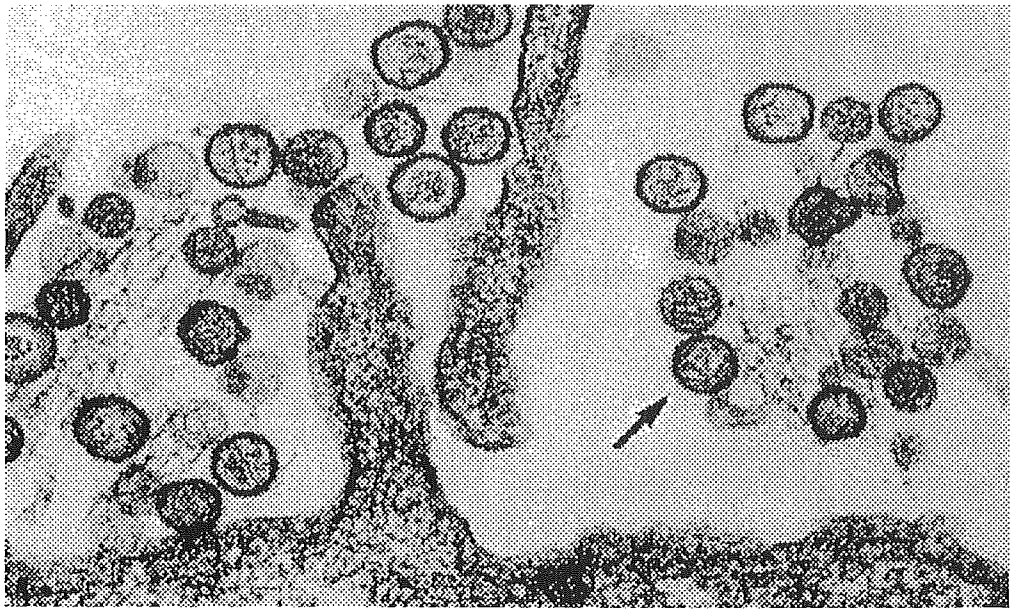

図2　シンノンブレウイルスの電子顕微鏡写真
ハンタウイルス肺症候群（HPS）の病原ウイルスであるシンノンブレウイルス
（写真提供：CDC）

表1　ヒトに病原性を有する各種ハンタウイルス

ウイルス型	宿主			分布	病型
	亜科	属	種　名（和　名）		
Hantaan	*Murinae*	*Apodemus*	*A. agrarius*（セスジネズミ）	アジア	HFRS
Dobrava			*A. flavicollis*（キクビアカネズミ）	ヨーロッパ	HFRS
Saaremaa			*A. agrarius*（セスジネズミ）	ヨーロッパ	HFRS
Amur			*A. peninsulae*（ハントウアカネズミ）	アジア	HFRS
Seoul		*Rattus*	*R. norvegicus*（ドブネズミ）	アジア	HFRS
Seoul			*R. rattus*（クマネズミ）	アジア	HFRS
Puumala	*Arvicolinae*	*Clethrionomys*	*C. glareolus*（ヨーロッパヤチネズミ）	ヨーロッパ	HFRS
Sin Nombre	*Sigmodontinae*	*Peromyscus*	*P. maniculatus*（シカシロアシマウス）	北アメリカ	HPS
Monongahehela			*P. maniculatus*（シカシロアシマウス）	北アメリカ	HPS
New York			*P. leucopus*（シロアシマウス）	北アメリカ	HPS
Bayou		*Oryzomys*	*O. palustris*（サワコメネズミ）	北アメリカ	HPS
Black Creek Canal		*Sigmodon*	*S. hispidus*（コットンラット）	北アメリカ	HPS
Andes		*Oligoryzomys*	*O. longicaudatus*（オナガコメネズミ）	南アメリカ	HPS
Lechiguanas			*O. flavescens*（キイロコメネズミ）	南アメリカ	HPS
Choclo			*O. fulvescens*（アカキコメネズミ）	南アメリカ	HPS
Laguna Negra		*Calomys*	*C. laucha*（ヨルマウス）	南アメリカ	HPS

（文献7を改変）

もしくは遺伝子型が報告され（表1）[7]，そのうちHFRSには6つの型が関与している。ウイルスの血清型，媒介動物および重篤度には強い相関があり（表1），死亡率の高い順にハンターン型（5～10%），ド

ブラバ型（5～10%），ソウル型（1%程度），プーマラ型（1%以下）となっている。アムール型は強毒型，サーレマ型は弱毒型とされるが，死亡率については明らかにされていない。また，HPSには少なくとも9つの型が関与していることが判明している。ウイルス遺伝子の塩基配列から得られた進化系統樹とげっ歯類の系統分類が一致することから，ハンタウイルスとげっ歯類は地質学的な長い時間をかけて共進化してきたものと考えられている[8]。

歴史と疫学

HFRSは，ユーラシア大陸の広い地域で発生が見られ，特に東アジアとヨーロッパ，ロシアなどで多発している。HFRSの最大の流行国は中国で，年間5～10万人の症例が報告されている[9]。その他にも韓国で年間数百人，ロシアやヨーロッパ各地で数千人の発生が見られる。感染げっ歯類は全く無症状のままウイルスを長期間保有し，糞尿中にウイルスを排出する。人はウイルスを含んだ粉塵を吸い込むことによって経気道的に感染する。人から人への水平感染は報告されていない。わが国では第二次大戦中，中国東北部において旧日本軍の間で約一万人の患者が発生して10%が死亡し，「流行性出血熱」と呼ばれた。国内では1960年代に大阪梅田駅周辺でドブネズミが感染源と疑われるHFRSの流行が発生し（119例中2例が死亡），「梅田熱」と呼称された[10]。さらに1970年から1984年まで，全国の大学や研究機関の実験動物施設で実験用ラットを介した実験室型の流行が発生した（126例中1例が死亡）[11]。現在は血清診断法の確立による感染動物の摘発淘汰が実施されたため患者発生は認められていない。しかし，ドブネズミや野ネズミを対象にした疫学調査で，全国20箇所の港湾地区で捕獲されたドブネズミや北海道のエゾヤチネズミがハンタウイルスに感染していることが明らかになった[12, 13]。幸い，人における流行は現在確認されていないが，何らかの原因で人とげっ歯類の接触機会が増加すれば，一般市民にもHFRSの再流行が起こる可能性がある。さらに，米国においてHPSが新たに出現したように，日本においても野ネズミが新型のハンタウイルスを保有しており，新型ウイルスに起因する新たなHFRSの流行が発生する可能性も否定できない。最近，我々は原因不明の肝炎患者にハンタウイルスの抗体を検出した[14]。現在，本ウイルス感染と肝炎発症との関係について検討中である。以上のように，日本の住居性ネズミや野ネズミも潜在的なHFRSの感染源として監視体制を強化する必要がある。

1993年，米国の南西部諸州で報告された原因不明の致死的な呼吸器感染症がこれまで知られていなかった新型のハンタウイルスの感染によって起こることが初めて明らかにされ，HPSと名づけられた[4]。その後の調査と研究により，原因ウイルスはシカシロアシマウス（図3）という北アメリカ大陸に固有のげっ歯類が病原巣動物であることが明らかになり[15]，シンノンブレウイルスと名付けられた。さらに，ウイルスは以前からシカシロアシマウスに保有されており，古くからHPSの散発的発生があったことも確認された。1993年のHPSの多発は地球規模の気象変動が原因だったと考えられている。1992年から1993年にかけて発生したエルニーニョ現象による降雨量の増加のため，北米大陸の南西部の砂漠地帯が緑地化したことが知られている。これにより，げっ歯類の爆発的な繁殖が起こり，

図3　シンノンブレウイルスの宿主であるシカシロアシマウス
シカシロアシマウス（*Peromyscus maniculatus*）は北米大陸のほとんどの地域に生息し，シンノンブレウイルスを媒介する。
（写真提供：CDC）

ヒトと感染げっ歯類との接触機会が増加したために，HPSが多発したものと考えられる。このように，環境の変化によって人獣共通感染症の発生状況が激変することがあるため，自然界における病原体の存続や伝播の様式を事前に解明しておくことが重要である。1993年の流行後も継続的なHPSの発生が見られており，2004年3月までに米国だけで363名の患者が報告されている。また，シカシロアシマウスの他にも北米大陸でHPSを媒介するげっ歯類が複数存在することが明らかになり，これらのげっ歯類がシンノンブレウイルスに近縁ではあるものの異なったハンタウイルスを保有していることも次第に明らかにされた。米国以外のアメリカ大陸でもHPSの発生が相次いでいる。2002年までにカナダ，アルゼンチン，チリ，パラグアイ，ウルグアイ，ブラジル，ボリビアなどから合計1,254名の患者が報告されている。HPSもHFRSと同様に感染げっ歯類の排泄物を吸い込むことによって感染が起こるが，HFRSと同様にヒトからヒトへの感染は起こらないと考えられていた。しかし，1996年アルゼンチンで発生した流行ではヒトからヒトへの空気感染が起こったことが判明した[16]。しかし，これ以後一度もヒトからヒトへのHPSの感染は報告されていないことから，このアルゼンチンの事例は非常にまれなことであると考えられている。

症状，診断及び検査法

HFRSの診断に有効な臨床症状を以下に列記する。1）突然の発熱と3～7日間の高熱の稽留とその後の解熱，2）蛋白尿（第6日頃をピーク），3）白血球減少（第3病日）の後増加（第6病日），4）血清GOT（glutamic oxaloacetic transaminase），GPT（glutamic pyruvic transaminase），LDH（lactate dehydrogenase），CPK（creatine phosphokinase）値の上昇，5）点状出血（上口蓋粘膜，躯幹部）などがあげられる。このように典型的なHFRSは腎臓障害や出血が主な症状となる。一方，HPSは頻呼吸を特徴とする呼吸困難が急速に出現し，重症例では入院8日目以内で死亡する。発症例の死亡率は約40%と言われている。死因は肺浮腫，肺水腫，低血圧およびショックで，胸腔内浸出液の貯留が顕著となる。HPS患者の血液像は白血球数の増加，血液濃縮と特に血小板の著しい減少が特徴である[17]。各種臓器の上皮細胞にウイルス抗原が確認されているが，中でも肺の上皮細胞に多量の抗原が分布する。しかし，ウイルスの局在部位に細胞障害が認められないことから，HPSの発症にはウイルス側の因子だけでなく，免疫担当細胞の活性化や血小板の消費による肺の微小血管の透過性の亢進など，患者の免疫反応が関係していると考えられている。HFRSもHPSも確定診断は抗体検出やPCRによるウイルス遺伝子の検出による。抗体検出はこれまで簡便で感度の高い方法として間接蛍光抗体法が広く使われてきた。最近では組換え蛋白を用いたウェスタンブロット法[18]やELISA(enzyme-linked immunosorbent assay)法[19]なども普及しつつある。わが国における診断キットは実験動物用のものが発売されているが[20]，人用のものは市販されていない。血清学的な確定診断にはIgM(immunoglobulin M)抗体の検出か，急性期と回復期のペア血清で抗体価の上昇を確認することが必要となる。抗体価は数年以上高い価を維持する。

予防方法

ハンタウイルス感染症はげっ歯類によって媒介されることから，まずげっ歯類集団において抗体調査を行って，流行地と病原巣動物を特定することが予防対策上重要である。流行地ではげっ歯類を人に近づけないことが最大の対策となる。すなわち，ネズミの駆除や衛生的な環境整備（ネズミの餌となるようなものを長期間保存しない，残飯などを放置しない）などに心懸けるべきである。また，HPSでは長期間使用しなかった家屋の清掃などで感染しやすいことがわかっているので，清掃時にはマスクや手袋の着用，粉塵発生の防止などに心懸ける必要がある。ワクチンは中国や韓国で不活化ワクチンが製造され，実用化されている。組換え蛋白を抗原としたワクチンやDNAワクチンなどの開発が米国等で試みられているが，実用化されていない。

治療法

抗ウイルス剤のリバビリンの有効性が試験的に検討されているが，主に対症療法による治療が行われる。

HFRSでは解熱前後におこる低血圧性ショックが主要な死亡原因となるので，厳重な安静が必要とされる。わが国ではワクチンが実用化されていないため，対症療法以外の有効な予防・治療法がない。患者発生時に迅速な対応が可能なように，人用の診断キットや抗ハンタウイルス剤の開発が重要な課題である。

わが国におけるハンタウイルス感染症発生の可能性

これまでげっ歯類媒介性のハンタウイルス感染症について概説してきた。他の危険度の高い新興・再興感染症と同様，ハンタウイルス感染症の発生は本来げっ歯類の生息域に人間が新たに侵入したり，人間の生活環境でげっ歯類が生息数を増したり，あるいは，温暖化などの影響で感染げっ歯類が大繁殖したことが原因と考えられる。したがって，今後，人間の活動様式や自然環境の変化によって，げっ歯類と人との接触の機会が増加すれば，日本においてもハンタウイルス感染症の突発的発生がいつ何時起こっても不思議ではない状況にあると考えられる。

わが国では感染例が極めてまれであるため，本症は外来性感染症でウイルス自体が日本に存在しないかのように錯覚されやすい。しかし，北海道の広い範囲でエゾヤチネズミが本ウイルスに感染しているばかりでなく[13]，日本各地の港湾地区でも陽性ドブネズミが検出されている[12]。これらのウイルスはいずれもHFRSの原因ウイルスに極めて近縁である[21, 22]。さらに，ハンタウイルスに対する抗体も低率ではあるがわが国のヒトにおいて検出されている。したがって，ハンタウイルスの感染はわが国においても発生していることが示唆される。また，極東ロシアや中国ではハンターンウイルスの他にも人に重篤なHFRSを引き起こすアムールウイルスと呼ばれるハンタウイルスが存在し，本ウイルスがハントウアカネズミを病原巣動物として存在することが遺伝子解析の結果から明らかになった（図4，5）[23]。したがって，これらの地域で野外活動する予定の旅行者はHFRSに感染する可能性がある。また，これらの地域からのげっ歯類の侵入に対してさらに対策を強化する必要がある。日本国内にはHPSを媒介するげっ歯類が生息していないことから，幸いこれまでにHPSの患者発生は認められていない。しかし，南北アメリカ大陸にはHPSの媒介動物が広く分布していることから，アメリカ大陸で野外活動を行う人はHPSの感染に注意を払う必要がある。

おわりに

財務省の貿易統計によれば平成14年には約75万匹のげっ歯類がわが国に輸入されていた。このうち人獣共通感染症を媒介する可能性のある野生げっ歯類は推定で約5万匹ほどであったと考えられる。これまではラッサ熱やペストの媒介動物であるマストミス，およびペストと野兎病を媒介するプレーリードッグが輸入禁止となっていたが，その他の種類については法的な規制が存在しなかった。しかし，平成15年に感染症法が改正されたことから，野生げっ歯類の輸入に対して

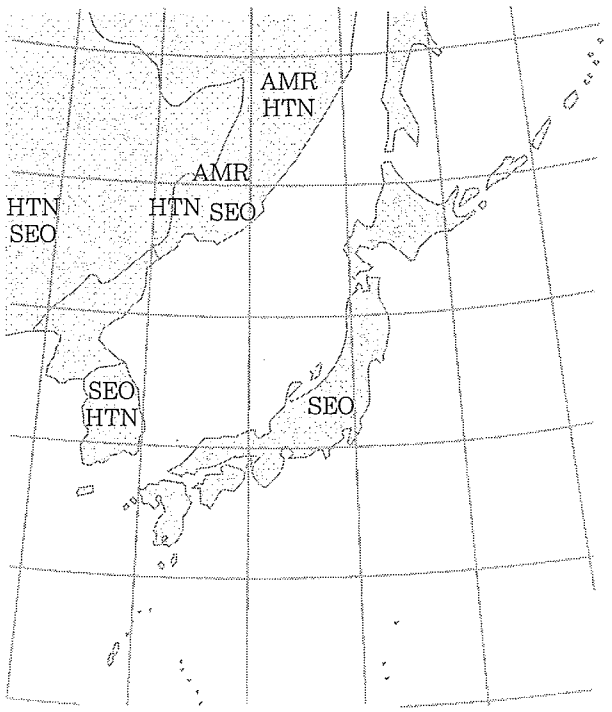

図4　東アジアにおける病原性ハンタウイルスの分布

極東ロシア，中国，および韓国では重症型HFRSの原因ウイルスであるハンターンウイルスとアムールウイルスが分布している。中等度の病原性を示すソウルウイルスは大陸と日本に分布している。

HTN：ハンターンウイルス，AMR：アムールウイルス，SEO：ソウルウイルス

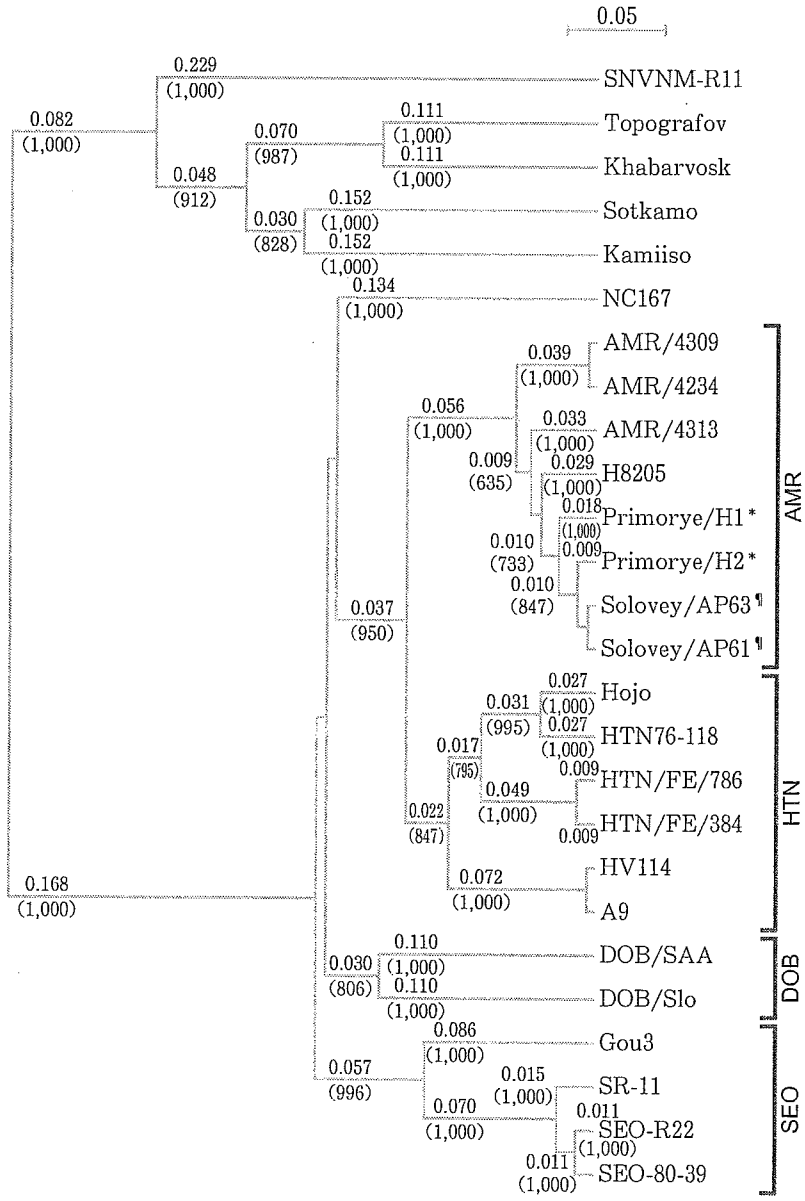

図5　ハンタウイルスのM遺伝子の系統樹解析

ウラジオストックのHFRS患者から検出されたウイルス（*）とハントウアカネズミから検出されたウイルス（¶）がアムールウイルスと共通の系統に属している。

HTN：ハンターンウイルス，AMR：アムールウイルス，DOB：ドブラバウイルス，SEO：ソウルウイルス

も法的な規制が行われるようになり，わが国への野生げっ歯類の輸入には輸出国側の厳格な検査が義務付けられることになった。しかし，船舶などに紛れ込んで日本に侵入する外来性のげっ歯類も存在すると考えられることから，港湾地域でのげっ歯類の監視活動の強化が望まれる。今後，日本国内に存在するハンタウイルスによるヒトの感染状況を明らかにするために，全国規模の疫学調査を行う必要があると考えられる。