Table 3. Comparison of sequence similarity (%) among the large noncoding regions (*LNRs*) of *L. akamushi* (La), *L. deliense* (Ld), *L. fletcheri* (Lf), and *L. pallidum* (Lp)

	LaLNR#1	LaLNR#2	LdLNR#1	LdLNR#2	LflLNR#1	LflLNR#2	LpLNR#1	LpLNR#2	LpLNR#3
LaLNR#2	**99**[a]								
LdLNR#1	76[b]	73							
LdLNR#2	74	73	**99**						
LflLNR#1	75	74	69	**69**					
LflLNR#2	75	75	68	69	**99**				
LpLNR#1	63	61	57	58	57	**57**			
LpLNR#2	63	61	56	57	57	57	**92**		
LpLNR#3	64	62	56	57	57	57	**94**	**98**	
LpLNR#4	64	62	57	58	57	58	**96**	**97**	**99**

[a] Values in boldface are similarities between two *LNRs* of the same species.
[b] Values in normal font are between two *LNRs* of different species.

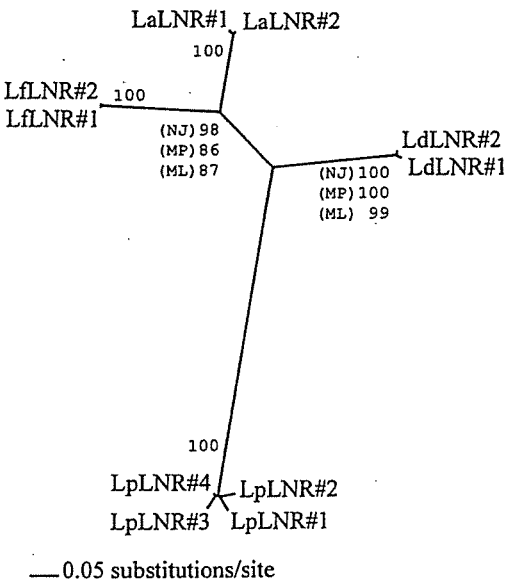

Fig. 4. An unrooted neighbor-joining (NJ) tree inferred from the nucleotide sequences of the large noncoding regions (*LNRs*) of *Leptotrombidium akamushi* (La), *L. deliense* (Ld), *L. fletcheri* (Lf), and *L. pallidum* (Lp). The maximum-likelihood (ML) and maximum-parsimony (MP) trees (not shown) have the same topologies as the NJ tree except for the grouping among the four *LNRs* of *L. pallidum*. Percentage bootstrap support (1000 replicates) is shown near each branch. The three branches that have only one value, 100, have 100% bootstrap support from all three methods of analysis: NJ, ML, and MP.

in the ancestral type of mt genome of the arthropods, and then to four *LNRs* in the type II genome; but it requires *three duplications and two deletions* to account for the evolution of four *LNRs* in the type II genome from one *LNR* in the ancestral type of mt genome of the arthropods, and then to two *LNRs* in the type I genome (Fig. 5C). Second, it requires *two duplications and two deletions* to account for the evolution of the arrangement, *rrnL-rrnS*, in the type I genome from the arrangement, *rrnS-V-rrnL*, in the ancestral type of mt genome of the arthropods, and then to the arrangements, *rrnL-rrnS* and *rrnL-PrrnS*, in the type II genome; but it requires *two duplications and three deletions* to account for the evolution of the arrangements, *rrnL-rrnS* and *rrnL-PrrnS*, in the type II genome from the arrangement, *rrnS-V-rrnL*, in the ancestral type of mt genome of the arthropods, and then to the arrangement, *rrnL-rrnS*, in the type I genome (Fig. 5D). Third, the inference that the type II genome evolved from the type I genome does not require any assumption about the phylogeny among the four *Leptotrombidium* species (Fig. 5A). However, the inference that the type I genome evolved from the type II genome requires the assumption that *L. akamushi*, *L. deliense*, and *L. fletcheri* are more closely related to each other than either of them is to *L. pallidum* (Fig. 5B). Otherwise, it would be difficult to explain why *L. akamushi*, *L. deliense*, and *L. fletcheri* had exactly the same deletions (Figs. 5C and D). There is no evidence so far for this assumption, although it is not necessarily wrong.

On the basis of the proposal above, we were able to address the second question: What mechanisms of gene rearrangement were likely involved in the evolution of the type of mt genome present in *L. pallidum* from the type of mt genome present in *L. akamushi*, *L. deliense*, and *L. fletcheri*? Several scenarios may be speculated but the most parsimonious one, in our view, is that two mechanisms of gene rearrangement were involved: (1) tandem duplication of a section of mt genome followed by random deletion of excess genes and (2) nonhomologous intergenome recombination. First, the gain of *LNR#3* between *Y* and *rrnL#1* in *L. pallidum* and the translocation of *Q* from between *Y* and *rrnL* in *L. akamushi*, *L. deliense*, and *L. fletcheri* to between *rrnS* and *LNR#1* in *L. pallidum*, i.e., from *Y-Q-rrnL-rrnS-LNR#1* to *Y-LNR#3-rrnL#1-rrnS-Q-LNR#1*, were generated by two events of tandem duplication of a section of genome followed by random deletion of excess genes (Fig. 1A). Tandem duplication followed by random deletion is a well-documented mechanism for mt gene rearrangement (see Moritz and Brown 1986; Boore 2000). The change from *Y-Q-rrnL-rrnS-*

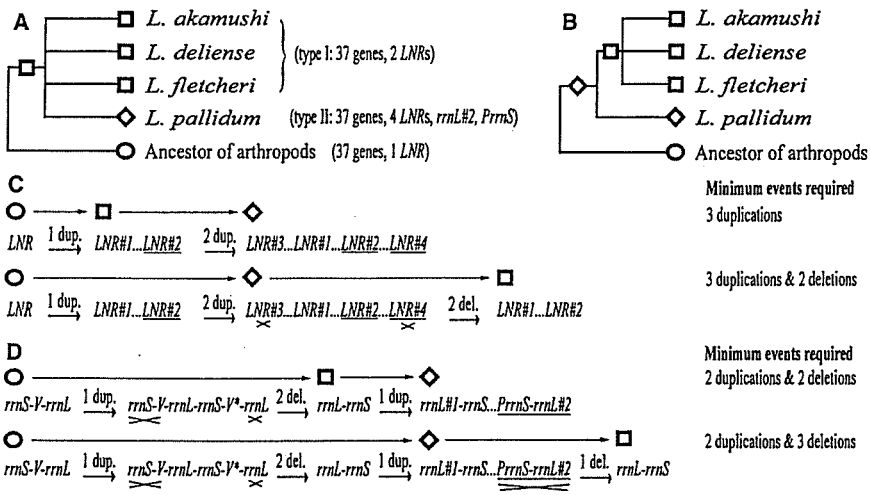

Fig. 5. Evolution of the two types of mitochondrial (mt) genomes of the *Leptotrombidium* species. Circles represent the ancestral type of mt genome of the arthropods, squares represent the type I genome of the *Leptotrombidium* species, and diamonds represent the type II genome of the *Leptotrombidium* species. **A** shows the inference that the type II genome evolved from the type I genome, and **B** shows the opposite inference. **C** and **D** illustrate the minimum events required to account for the evolution of the large noncoding region (*LNR*) and the arrangement of *rrnL* and *rrnS*, respectively, under the two inferences shown in A and B. *L. Leptotrombidium*; dup., duplication(s); del., deletion(s). See the legend to Fig. 1 for the abbreviation of gene names. Arrow lines indicate the direction of evolution. X symbols indicate deletions. Genes underlined and not underlined have opposite orientations of transcription in an mt genome. Genes linked by a hyphen are next to each other; genes linked by dots are separated from each other. The numbering and the order of *LNRs* in C and D are consistent with those in Fig. 1; so is the order of *rrnL#1-rrnS* and *PrrnS-rrnL#2*. Asterisks denote that *V* is not immediately downstream of *rrnS* in the mt genomes of *Leptotrombidium* species (see Fig. 1).

LNR#1 to *Y-LNR#3-rrnL#1-rrnS-Q-LNR#1* bears four typical characteristics of this mechanism: (1) a section of a genome (*LNR*) was duplicated; (2) neighbor genes (*Q* and *rrnL-rrnS*) swapped positions; (3) no changes in the orientation of transcription occurred; and (4) the section involved in the change contains *LNR* and, thus, is at the hot spot for tandem duplication (Boore and Brown 1998). In the case of this change, there is no need to invoke any other mechanisms, i.e., tandem duplication followed by transcription-orientation-dependent deletion of excess genes or nonhomologous intra- or intergenome recombination (see also Introduction), because there is no sign suggestive of these mechanisms, e.g., change of the orientation of transcription or distant translocation of single genes. Second, the gain of the section *PrrnS-rrnL#2-LNR#4* between *LNR#2* and *W* in *L. pallidum*, in addition to having *LNR#3-rrnL#1-rrnS* between *Y* and *Q* (genes underlined and not underlined have opposite orientations of transcription in the genome), was generated by a single event of nonhomologous *inter*genome recombination (Fig. 1B). The only alternative explanation is that tandem duplication generated *LNR#3-rrnL#1-rrnS-LNR#4-rrnL#2-PrrnS* first and then nonhomologous *intra*genome recombination moved and inverted *LNR#4-rrnL#2-PrrnS* to be between *LNR#2* and *W*. This explanation requires one event of tandem duplication and one event of nonhomologous *intra*genome recombination and, thus, is less parsimonious than the explanation of a single event of nonhomologous *inter*genome recombination (see also Shao et al. 2005a).

Concerted Evolution of Duplicate or Quadruple Large Noncoding Regions in Each Leptotrombidium *Species*

Shao et al. (2005b) showed that duplicate *LNRs* (also called control regions) in the mt genome of Metazoa tend to evolve in concert rather than independently. For all other species of Metazoa that have duplicate *LNRs*, the two *LNRs* in an mt genome have the same orientation of transcription. However, for *Leptotrombidium* species, the two or four *LNRs* in an mt genome have opposite orientations of transcription (Fig. 1). Yet two lines of evidence indicate that the *LNRs* of each *Leptotrombidium* species have also evolved in concert. First, the nucleotide sequences of the two or four *LNRs* of a *Leptotrombidium* species are substantially more similar to each other (92–99%; Table 3) than they are to the *LNRs* of other *Leptotrombidium* species (56–76%). Second, the two or four *LNRs* of each species always clustered together with substantial bootstrap support in our phylogenetic analyses (86–100%), regardless of whether the NJ, ML, or MP method was used (Fig. 4). Two mechanisms may account for the concerted evolution of duplicate *LNRs*: tandem duplication of a section of genome, followed by random deletion of excess genes, and gene conversion (Kumazawa et al. 1998). The former, however, cannot account for the concerted evolution

of duplicate *LNRs* that have opposite orientations of transcription. The latter, gene conversion, can account for the concerted evolution of *LNRs* that have opposite orientations of transcription, such as those in the *Leptotrombidium* species. Gene conversion is a type of homologous recombination. For gene conversion to occur, the two *LNRs* of an mt genome need to be physically close to each other, and to align with each other in the same orientation of transcription, so that a Holliday structure can form (Kumazawa et al. 1998). It is known that in the fruit fly, *Drosophila melanogaster*, the mt genomes exist in two distinct and stable superhelical forms: one has few turns, whereas the other has many turns (Rubenstein et al. 1977). Such superhelical forms of the mt genome make it possible that a Holliday structure can form between two *LNRs* of an mt genome, regardless of whether the two *LNRs* have the same or opposite orientations of transcription.

In conclusion, we found that two types of mt genome exist in the genus *Leptotrombidium*: one in *L. akamushi* and *L. deliense*, and probably in *L. fletcheri* too; the other in *L. pallidum*. It is more likely that the type of mt genome present in *L. pallidum* (with 37 genes typical of Metazoa, four *LNRs*, an extra *rrnL*, and a *PrrnS*) evolved from the type of mt genome present in *L. akamushi*, *L. deliense*, and *L. fletcheri* (with 37 genes and two *LNRs*) than vice versa. The simplest scenario for this evolution involves two mechanisms: tandem duplication of a section of genome followed by random deletion of excess genes and nonhomologous intergenome recombination. A third mechanism, gene conversion, is required to account for the concerted evolution of the duplicate or quadruple *LNRs* that have opposite orientations of transcription in each species of *Leptotrombidium*. The *Leptotrombidium* chigger mites thus provide evidence for the action of multiple molecular mechanisms in the evolution of gene content and gene arrangement in the mt genomes of Metazoa.

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