Letter to the Editor

The Evolutionary Position of Dormice (Gliridae) in Rodentia Determined by a Novel Short Retroposon

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Exploring the genealogical relationships between taxa or building phylogenetic trees is usually based on comparison of functional characters (either morphological ones or the sequences of genes and proteins). However, such characters are affected by various evolutionary processes (such as convergence), which complicates the analysis and can lead to erroneous inferences. (Examples of contradictory conclusions can be found in Hartenberger 1985; Cao, Okada, and Hasegawa 1997; Springer et al. 1997; Rougier and Novacek 1998).

Use of characters beyond selective pressure (phylogenetic markers) could clearly provide more reliable results. This approach has recently been applied by Shimamura et al. (1997), who used insertion of short interspersed retroposons (SINEs) at specific sites in the genome as landmarks in mammalian evolution and, as a result, showed that whales form a clade within even-toed ungulates.

We have developed a similar method using another binary qualitative character, presence of short retroposon families in the genomes, to provide landmarks of host evolution. Using this approach, we determined the phylogenetic distribution of different SINE families by PCR with family-specific primers followed by hybridization, and we have confirmed the generally accepted phylogenetic relationships between mice (Muridae), hamsters (Cricetidae), and mole rats (Spalacidae), as well as between jerboas (Dipodidae) and birch mice (Zapodidae) (Serdobova and Kramerov 1998). Here, we applied this approach to re-examine the traditional view on evolutionary relationships between dormice (Gliridae) and myomorphous rodents.

SINEs are 80–400-bp genomic repeats, apparently originating from RNA genes. They replicate in the genome via RNA polymerase III transcription and reverse transcription (Jagadeeswaran, Forget, and Weissman 1981). We found a novel class of short retroposons in the dormouse genome, composed of two units similar to other rodent SINEs, B1 (Krayev et al. 1980) and ID (Sutcliffe et al. 1982) (fig. 1*A*). We detected this element (designated B1-dID) in rodent genomes by PCR with primers GCAYRCCTTTAATCCCAG and CTGGGGATKGAACCCAGRG, complementary to conservative regions in the B1- and ID-like monomers, respectively (fig. 1*A*). After agarose gel electrophoresis, the PCR products were transferred on a nylon filter and hybridized with a 47-nt fragment of the ID-like unit of the dormouse B1-dID (fig. 1*A*) under relaxed and stringent conditions. PCR and hybridization conditions were described elsewhere (Serdobova and Kramerov 1998).

Figure 1*B* demonstrates ~185-bp PCR products amplified from the genomes of dormice and squirrels, as well as beavers, porcupines, and guinea pigs, but not from those of myomorphous rodents (mice, voles, mole rats, birch mice, and jerboas). Note the 10–1,000 excess of the DNA template required for similar band intensity for beavers, porcupines, and guinea pigs, reflecting a significant difference between the number of B1-dID copies in their genomes as compared with those of dormice and squirrels. Hybridization with the dormouse ID-like probe under relaxed and stringent conditions suggests a close similarity between the dormouse and squirrel B1-dIDs (designated gsB1-dID), and a more distant similarity between these retroposons and B1-dIDs from beavers, porcupines, and guinea pigs. (The smeared fragments represent B1 and ID interelement PCR products rather than B1-dID.) Accordingly, the B1-dID sequences that we cloned from the forest dormouse, the palm squirrel, and the marmot were much more similar than were those from the beaver, the porcupine, and the guinea pig (data not shown; EMBL/GenBank accession numbers Y16204–Y16223).

Presence of SINEs in genomes would indicate a common origin of the host species if three conditions are met at the same time: all SINEs of a particular family (1) had a common origin, (2) were distributed by vertical transmission, and (3) were not eliminated from the genome. First, it seems quite unlikely that the fusion of B1 and ID units, as well as certain specific features of most B1-dID sequences (e.g., a 19-nt deletion in the ID-like unit indicated in fig. 1*A*), occurred independently in different taxa. Second, vertical transmission is the only known way of SINE distribution (Deininger and Batzer 1993). Closer examination of the only documented SINE horizontal transfer (Kordis and Gubensek 1995) suggests that this exotic element originates from LINE (Kordis and Gubensek 1998), which is not necessarily distributed horizontally (Malik and Eickbush 1998). Third, there are no data on elimination of SINE families from genomes; for instance, the MIR element was preserved in the genome during the continuance of mammalian evolution (Smit and Riggs 1995).

Hence, according to the B1-dID distribution, dormice (Gliridae) share a common origin with squirrels (Sciuridae) rather than with the myomorphous rodents. This result differs from the phylogeny based on certain morphological (Wood 1965; Romer 1966; Wahlert, Sawitzke, and Holden 1993) and molecular data sets (Sarich 1985), but it agrees with other morphological (Carrol

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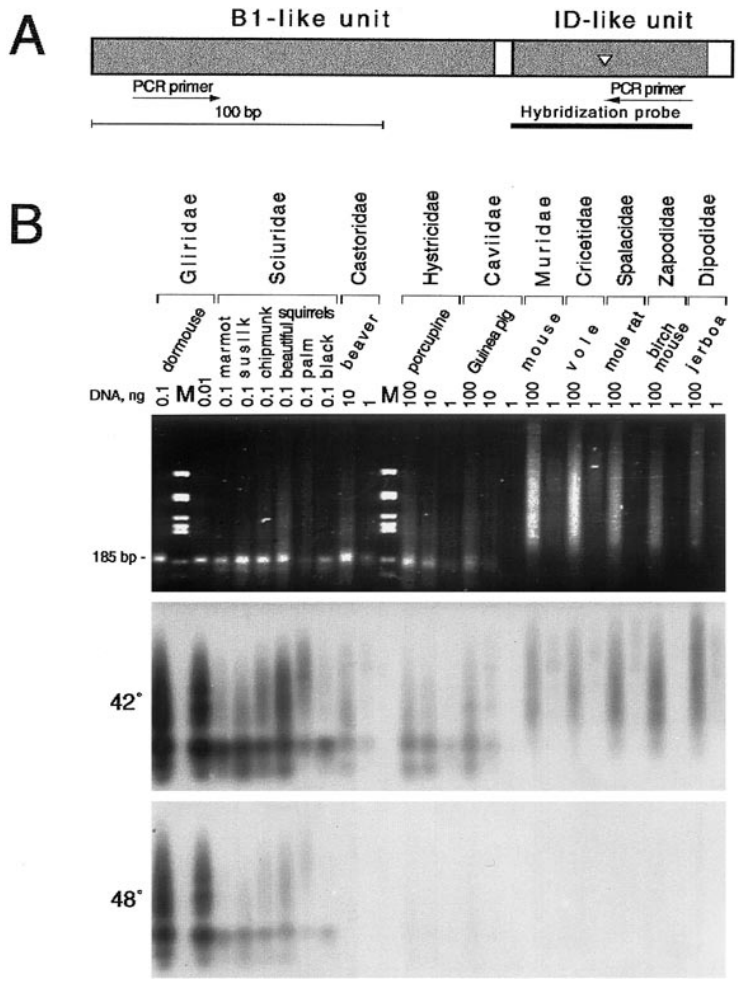

FIG. 1.—*A,* B1-dID retroposon structure. Open rectangles indicate A-rich tails, while the open triangle points to the specific 19-nt deletion. *B,* PCR (upper panel) and PCR-hybridization analysis of B1-dID in rodent genomes with relaxed (42°C) and stringent (48°C) washing conditions (lower panels). The amounts of genomic DNA are indicated above the lanes; dormouse, *Dryomys nitedula*; marmot, *Marmota caudata*; suslik, *Citellus fulvus*; chipmunk, *Tamias sibiricus*; beautiful squirrel, *Callosciurus caniceps*; palm squirrel, *Menetes berdmorei*; black squirrel, *Sciurus carolinensis*; beaver, *Castor fiber*; porcupine, *Hystrix leucura*; guinea pig, *Cavia porcellus*; mouse, *Mus musculus*; vole, *Microtus daghestanicus*; mole rat, *Spalax microphtalmus*; birch mouse, *Sicista tianschanica*; jerboa, *Allactaga major*; the corresponding families are indicated above; M, size marker (pGEM7zf/*Hae*III).

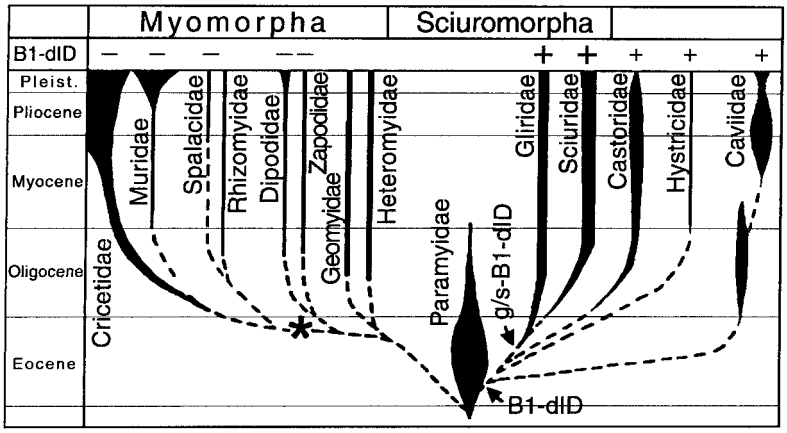

FIG. 2.—Partial evolutionary tree of rodent families and the distribution of B1-dID. The asterisk indicates the traditional position (Romer 1966) of the dormouse branchpoint; presence and absence of the element is indicated by large (high copy number) or small (low copy number) "+" and "−" symbols, respectively; arrows point to the presumptive appearances of the B1-dID elements and their Gliridae/Sciuridae variants.

1988) and molecular data (Nedbal, Honeycutt, and Schlitter 1996).

On the other hand, the data obtained demonstrate that the Myomorpha lineage differentiated from other rodents before their branching. This conclusion contrasts with the phylogeny proposed by Hartenberger (1985) and supports that of Nedbal, Honeycutt, and Schlitter (1996) and Reyes, Pesole, and Saccone (1998).

Figure 2 shows a partial tree of rodent evolution (Romer 1966), modified as a result of our analysis, in which the dormouse branch is transferred from the myomorphous rodents to squirrels, and all B1-dID-positive families are placed together.

In general, although short-retroposon analysis has too low a resolution to independently construct a phylogeny, it offers a highly reliable and simple test of the evolutionary relationships between taxa.

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