Evolution of Sea Urchin Retroviral-Like (SURL) Elements: Evidence from 40 Echinoid Species

Pedro Gonzalez[1] *and H. A. Lessios*

Smithsonian Tropical Research Institute, Balboa, Panama

We conducted a phylogenetic survey of sea urchin retroviral-like (SURL) retrotransposable elements in 33 species of the class Echinoidea (sea urchins, sand dollars, and heart urchins). A 263-bp fragment from the coding region of the reverse transcriptase (RT) gene was amplified, cloned, and sequenced. Phylogenetic relationships of the elements isolated from independent clones, along with those from seven additional echinoid species obtained earlier by Springer et al., were compared with host phylogeny. Vertical transmission and the presence of paralogous sequences that diverged prior to host speciation can explain most of the phylogenetic relationships among SURL elements. Rates of evolution were estimated from cases in which SURL and host phylogenies were concordant. In agreement with conclusions reached previously by Springer et al., average rates of synonymous substitution were comparable with those of single-copy sea urchin DNA. High ratios of synonymous to nonsynonymous substitution suggest that the RT of the elements is under strong purifying selection. However, a high proportion (~15%) of elements with deleterious frameshifts and stop codons and an increase of the ratio of synonymous to nonsynonymous substitutions with divergence time show that in the short term this selection is relaxed. Despite the predominance of vertical transmission, sequence similarity of 83%–94% for SURL elements from hosts that have been separated for 200 Myr suggests four cases of apparent horizontal transfer between the ancestors of the extant echinoid species. In three additional cases, elements with identical RT sequences were found in sea urchin species separated for a minimum of 3 Myr. Thus, horizontal transfer plays a role in the evolution of this retrotransposon family.

Introduction

Mobile genetic elements (transposable elements, TEs) have been found in the genomes of all taxonomic groups that were studied for this purpose (Finnegan 1989; McDonald 1993, 1995; Kunze, Saedler, and Lönnig 1997). Class I elements are related to retroviruses and transpose though the reverse synthesis of DNA from template RNA; class II elements move in the genome through direct DNA-to-DNA transposition without an RNA intermediary. Class I elements are known as retrotransposons and include TEs with and without long terminal repeated sequences (LTRs). Retrotransposons with LTRs include the Ty3/*gypsy* group and the Ty1/*copia* group. Springer, Davidson, and Britten (1991) have described a family of transposons in sea urchins that belong to the Ty3/*gypsy* group. The *pol* region of these elements is composed of protease, reverse transcriptase (RT), RNAaseH and integrase domains. Because this group of retrotransposons is very similar to retroviruses, they were named ''sea urchin retroviral-like'' (SURL) elements.

Transposition of TEs is responsible for most spontaneous mutations in *Drosophila* (Green 1988; Smith and Corces 1991; Charlesworth, Sniegowski, and Stephan 1994), and it can have serious fitness consequences (MacKay 1986; Eanes et al. 1988; Dombroski et al. 1991; Wallace et al. 1991; Woodruff 1993; Kidwell and Lisch 1997; Kazazian and Moran 1998). Certain kinds of elements are responsible for hybrid dysgenesis (Rubin, Kidwell, and Bingham 1982; Kidwell 1983; Louis and Yannopoulos 1988; Scheinker et al. 1990). They also appear to cause chromosomal deletions, duplications, inversions, and translocations (Finnegan 1989; Kidwell and Lisch 1997). The insertion of TEs can alter expression of the host's genes (McDonald 1990, 1993, 1995; Kidwell and Lisch 1997) and lead to novel enzymatic functions (Britten 1996, 1997). For these reasons, some authors have proposed that TEs could be involved in the formation of new species (Rose and Doolittle 1983; McDonald 1989, 1990) and act as catalysts of organismic evolution (McDonald 1990, 1995; Britten 1996). Additional interest in the study of TEs is generated by the possibility that they may be useful in the retrieval of phylogenetic information that is difficult to infer from conventional DNA sequence comparisons (Murata et al. 1993; Usdin et al. 1995; Cook and Tristem 1997; Takahashi et al. 1998; Verneau, Catzeflis, and Furano 1998).

A major point of controversy about TE evolution is the possible role of horizontal transmission between phylogenetic lines. Arguments for and against horizontal transmission have been the subject of a long debate (reviews in Kidwell 1992, 1993; Capy, Anoxolabéhère, and Langlin 1994; Cummings 1994). Convincing examples of horizontal transfer to date have been limited to those pertaining to class II elements (Daniels et al. 1990; Maruyama and Hartl 1991; Simmons 1992; García-Fernández et al. 1993; Clark, Maddison, and Kidwell 1994; Lohe et al. 1995; Robertson and Lampe 1995; Robertson 1997; Clark, Kim, and Kidwell 1998). Evidence for horizontal transmission of retrotransposons (Mizrokhi and Mazo 1990; Konieczny et al. 1991; Flavell 1992; McHale et al. 1992; Alberola and de Frutos 1993*a*, 1993*b*; Monte, Flavell, and Gustafson 1995) is more

[1] Present address: Duke Eye Center, Durham, North Carolina.

Abbreviations: LTR, long terminal repeat; ORF, open reading frame; RT, reverse transcriptase; SURL, sea urchin retroviral-like; TE, transposable element.

Key words: retrotransposons, sea urchins, reverse transcriptase, horizontal transfer, vertical transmission.

Address for correspondence and reprints: H. A. Lessios, Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama. E-mail: lessiosh@naos.si.edu.

Mol. Biol. Evol. 16(7):938–952. 1999

equivocal (Kidwell 1993; Capy, Anoxolabéhère, and Langlin 1994; Lathe et al. 1995; Springer et al. 1995; Krishnapillai 1996; Flavell et al. 1997). This is somewhat surprising, because their similarity to retroviruses, the discovery of an *env* open reading frame (ORF) in Ty3/*gypsy* (Springer and Britten 1993), and the experimental demonstration of infective properties in this retrotransposon family (Kim et al. 1994; Bucheton 1995) would lead to the expectation that jumping across phylogenetic lines might be easier in class I than in class II elements. However, a number of studies have concluded that the phylogenetic signatures of several retrotransposable elements did not require the hypothesis of horizontal transfer to be reconciled with the phylogeny of the hosts (e.g., VanderWiel, Voytas, and Wendel 1993; Lathe et al. 1995; Monte, Flavell, and Gustafson 1995; Springer et al. 1995; Usdin et al. 1995; Lathe and Eickbush 1997; McAllister and Werren 1997).

The question of apparent horizontal transmission of retrotransposons is complicated by uncertainties concerning their rates of evolution. It has been suggested that transposition that depends on error-prone reverse transcription may accelerate rates of mutation and divergence between active elements (Doolittle et al. 1989; Flavell 1992; Alberola and de Frutos 1993*a*), such that divergence between vertically transmitted active TEs should exceed divergence between host DNAs. However, Springer et al. (1995) estimated that the rates of evolution at synonymous sites of SURL elements are similar to those of nuclear genes; Eickbush et al. (1995) reached the same conclusion concerning R1 and R2 elements.

Eucaryote groups with well-resolved phylogenies provide an opportunity to study TE evolution with a minimum of complications arising from uncertainties regarding host phylogenies. The phylogeny of the echinoderm class Echinoidea has been extensively studied by morphological and molecular analysis of extant species, as well as by reference to the fossil record (Durham 1966; Jensen 1981; Smith 1981, 1984, 1988, 1989, 1992; Smith, Lafay, and Christen 1992; Smith and Littlewood 1994; Littlewood and Smith 1995). Springer et al. (1995) analyzed SURL elements in 10 echinoid species and found no evidence of horizontal transfer. The disagreements between phylogenies of transposons and those of hosts could be explained as arising from paralogous duplications that had occurred before the separation of the species. Springer et al. (1995) stressed the need for dense sampling of clades to distinguish between events of vertical and horizontal transmission. Here we present phylogenetic relationships of SURL elements in 40 species of echinoids, 30 of them not included in the study by Springer et al. (1995). We compare SURL phylogeny with host phylogeny and discuss the results with regard to rates of SURL evolution and the importance of horizontal transmission.

Materials and Methods

Table 1 lists the species of sea urchins from which SURL sequences were successfully amplified and presents their currently accepted classifications. Genomic DNA was extracted from one to three individuals per species from either gonad or muscle tissue. The DNA was subjected to polymerase chain reaction (PCR) amplification. Oligonucleotide primers for PCR amplification were surl-f: 5′-GGNGTNAAHACNATGATGGAYGA-3′ (identical to primer DD of Springer et al. [1995] but with three extra nucleotides [GGN] in the 5′ end), and surl-r: 5′-ARRTYNGGDTARAAYTTNSCYTGRTA-3′. The 3′ end of surl-r coincides with position 3959 of the complete *Tripneustes gratilla* SURL sequence of Springer, Davidson, and Britten (1991) (GenBank accession number M75523). In order to obtain as many elements as possible in each amplification reaction, both primers were designed for well-conserved regions of the RT gene (Springer, Davidson, and Britten 1991; Eickbush 1994), and with the necessary degeneracy to include each possible codon for a given amino acid. Amplification reactions were generally carried out in 50-μl volumes. Reaction conditions were 35 cycles of template denaturation for 30 s at 94°C, primer annealing for 130 s at 42°C, and primer extension for 30 s at 72°C. PCR products were separated in 2% low-melting TAE-agarose and stained with ethidium bromide. Bands of the expected size were melted at 45°C and cloned with the pMOS*Blue* T-vector kit (Amersham), following the specifications of the manufacturer. Inserts from randomly selected white bacterial colonies were PCR- amplified using pUC universal primers, and their sizes were verified in agarose gels. Amplified inserts were sequenced on either a 373A or a 377 automatic sequencing machine (Perkin-Elmer/Applied Biosystems), with the same pUC universal primers used for amplification. Sequencing of each clone was done in only one direction, but the direction varied between clones. With short gaps necessary for alignment, this resulted in a datum matrix of 277 SURL RT sequences, each 263 bp in length. Thirty- eight additional sequences obtained by Springer et al. (1995) were kindly provided by M. Springer; from these, we used the same 263-bp region we sequenced for all other sea urchins, for a total number of 315 SURL sequences. The 202 unique sequences obtained by us have been deposited in GenBank under accession numbers AF112643–AF112844.

Sequences were aligned by eye using computer program SeqEd, version 1.0.3. (Applied Biosystems). Identical sequences from different clones derived from the same species of sea urchin were treated as a single element for phylogenetic analysis. Phylogenies were reconstructed with test version 4.0d61 of PAUP*, written by D. L. Swofford and used for this analysis with his permission. Calculations of molecular divergence were performed using SEQUENCER, a program written by B. D. Kessing.

Results and Discussion

Comparisons with Previously Published SURL Sequences and Intraspecific Variation

We sequenced SURL clones from three species that had also been used in the Springer et al. (1995) study

Table 1
Classification of Echinoids Included in this Study, Collection Locality, Number of Individuals Sampled (*N*), Total Number of Independent Amplifications of SURL Elements (*A*), Number of Clones Sequenced (*C*), Number of Unique Sequences Obtained (*S*), Mean Kimura (1980)–Corrected Percentage of Sequence Divergence Among Unique Sequences from the Same Host (K_2), and Range of Sequence Divergences Within a Host (*R*)

	Locality	*N*	*A*	*C*	*S*	K_2 (%)	*R* (%)
Subclass Cidaroidea							
Order Cidaroida							
Family Cidaridae							
*Eucidaris tribuloides***	Atlantic Panama	2	3	10	8	2.00	0.40–4.10
Eucidaris thouarsi	Pacific Panama	1	2	10	6	2.96	0.00–4.60
Eucidaris metularia	Guam	1	1	1	1	—	—
Hesperocidaris panamensis	Pacific Panama	1	1	5	5	20.00	3.00–45.50
Hesperocidaris dubia	Pacific Panama	2	2	11	9	7.09	1.60–10.50
Prionocidaris bispinosa	West Australia	1	3	14	14	2.18	0.40–4.60
Subclass Euechinoidea							
Order Echinothuroida							
Family Echinothuriidae							
Araeosoma leptaleum	Pacific Panama	1	3	15	10	2.28	0.40–7.90
Order Diadematoida							
Family Diadematidae							
Astropyga pulvinata	Pacific Panama	1	1	5	3	1.07	0.80–1.20
Astropya magnifica	Atlantic Panama	1	1	2	2	0.40	—
Diadema antillarum	Atlantic Panama and Puerto Rico	2	3	20	3	1.07	0.80–1.20
Diadema mexicanum	Pacific Panama and Pacific Mexico	2	3	30	2	0.80	—
Diadema setosum	Japan	1	1	5	5	1.30	0.40–2.50
Diadema savignyi	Samoa	1	1	4	4	1.73	0.40–2.40
Centrostephanus coronatus	Galapagos	1	1	5	3	1.63	1.20–2.50
Echinothrix diadema	Isla Coco, eastern Pacific	1	2	6	6	4.24	0.40–10.80
Order Pedinoida							
Family Pedinidae							
Caenopedina diomedeae	Pacific Panama	3	3	20	20	3.33	0.40–9.00
Cohort Echinacea							
Superorder Stirodonta							
Order Arbacioida							
Family Arbaciidae							
Arbacia stellata	Pacific Panama	1	2	2	2	0.80	—
*Arbacia punctulata***	Atlantic Honduras	2	2	10	6	3.68	0.50–7.30
Superorder Camarodonta							
Order Echinoida							
Family Echinometridae							
Subfamily Strongylocentrotidae							
*Strongylocentrotus purpuratus**	Pacific North America			4	4	3.55	0.90–5.80
*Strongylocentrotus droebachiensis**	Atlantic and Pacific North America			6	4	1.90	0.40–3.70
*Strongylocentrotus franciscanus**	Pacific North America			5	3	4.43	1.50–6.80
Subfamily Echinometridae							
Echinometra vanbrunti	Pacific Panama	1	1	8	7	2.85	0.80–5.10
Echinometra viridis	Atlantic Panama	1	1	8	8	3.28	0.40–5.80
Echinometra lucunter	Atlantic Panama	1	1	7	6	2.87	0.40–5.80
Echinometra mathaei	Hawaii	1	1	11	10	3.79	0.40–7.80
Caenocentrotus gibbosus	Galapagos	2	2	23	20	2.22	0.40–8.30
*Heliocidaris tuberculata**	Southeast Australia			1	1	—	—
*Heliocidaris etythrogramma**	South Australia			2	2	44.70	—
Subfamily Toxopneustidae							
Tripneustes depressus	Isla del Coco, eastern Pacific	1	1	5	4	8.72	6.60–12.00
Tripneustes ventricosus	Atlantic Panama	1	1	5	4	7.35	0.80–12.90
*Tripneustes gratilla**	Indo-Pacific			4	3	10.37	9.00–12.30
*Lytechinus variegatus**	Atlantic Panama	2	2	6	6	1.95	0.90–3.40

Table 1
Continued

	Locality	*N*	*A*	*C*	*S*	K_2 (%)	*R* (%)
Cohort Irregularia							
Order Clypeasteroida							
Family Clypeasteridae							
Clypeaster rosaceus	Atlantic Panama	1	1	5	3	8.13	4.10–10.40
Clypeaster subdepressus	Atlantic Panama	1	1	2	2	10.40	—
*Dendraster excentricus**	Pacific North America			1	1	—	—
Order Spatangoida							
Family Schizasteridae							
Paraster doederleini	Atlantic Honduras	1	1	5	5	3.04	1.60–5.30
Agassizea scrobiculata	Pacific Panama	1	1	8	8	3.29	0.80–5.00
Family Brissidae							
Meoma ventricosa	Atlantic Panama	1	1	8	7	2.14	0.40–4.50
Plagiobrissus grandis	Atlantic Panama	1	1	3	3	2.47	2.00–2.90
Brissopsis elongata	Atlantic Honduras	1	1	13	13	2.79	0.40–5.40

NOTE.—One asterisk indicates that SURL sequences were obtained by Springer et al. (1995); in these cases, ''locality'' indicates the range of the species. Two asterisks indicate that SURL sequences were independently obtained by both Springer et al. (1995) and the present study. The echinoid classification follows Littlewood and Smith (1995).

(table 1). The most similar sequences from species included in both studies showed Kimura (1980) two-parameter corrected differences of 0.4% in *Eucidaris tribuloides,* 0.8% in *Arbacia punctulata,* and 1.4% in *Lytechinus variegatus.* This high degree of similarity confirms that our PCR primers have amplified the same SURL subfamilies as those of Springer et al. (1995). It is also indicative of low SURL polymorphism between conspecific individuals of these sea urchin species. Although Springer et al. (1995) do not mention where the sea urchins in their study were collected, they are unlikely to have come from anywhere close to the same areas as those used in our study (table 1). Thus, it appears that SURL subfamilies are geographically widespread. For five additional species, we sequenced SURL elements from more than one individual. In *Diadema mexicanum,* only two sequences were identified in the amplification of 30 clones, and they are both found in each of the two individuals we sampled, one from Panama, the other from Mexico. In *Diadema antillarum,* one SURL sequence was found in two individuals, one from Panama, the other from Puerto Rico. The difference between the other two sequences, unique to each individual, was 0.8%, about equal to the average divergence between different elements found in the same individual (1.2%). In *Caenocentrotus gibbosus,* two sequences are shared between individuals; among the remaining 18, the within-individual divergence is 2.5% and the between-individual difference is 2.4%. In *Hesperocidaris dubia,* the average difference between elements from different individuals (7.3%) is only slightly larger than the difference between elements within the same individual (6.7%). In *Caenopedina diomedeae,* the only species for which elements from three individuals were sampled, the average difference between clones from different specimens (3.6%) is also comparable with that of clones from the same specimen (3.3%). Among the elements sequenced in this study, the largest Kimura-corrected distance between sequences from the same individual was in *Hesperocidaris panamensis,* in which one clone was 39.2%–45.5% different from all other clones. This is comparable with divergence found by Springer et al. (1995) between SURL elements from two different individuals of *Heliocidaris erythrogramma* (44.7%). The apparently low between-individual polymorphism in SURLs relative to the differences among elements occurring in the same individual suggests that the approach of assaying retrotransposons from only a few specimens from each sea urchin species is not likely to lead to serious errors in the comparison between SURL and host phylogenetic trees.

There was a striking difference between the numbers of different sequences obtained from different clones for each sea urchin species (table 1). In some, such as *Agassizea scrobiculata, Brissopsis elongata, C. diomedeae, Diadema savignyi, D. setosum, Echinometra viridis, Echinothrix diadema, L. variegatus, Paraster doederleini,* and especially *Prionocidaris bispinosa* (in which 14 clones were sequenced), each clone turned out to be unique. In many others, there were one or two identical sequences between clones, but the rest were distinct. In *D. antillarum,* however, 20 clones from a total of three separate amplifications from two individuals produced only three unique sequences. In *D. mexicanum,* only two unique sequences were found among 30 clones from three amplifications from two individuals. Clones with identical sequences could, of course, be the result of bias in the amplification of a few elements from the many that might exist in the genome because of differential affinity to the primers or because of unequal copy numbers. However, the primers were designed for very well conserved regions of the RT coding region, with the maximum possible degeneracy, and they appeared to be capable of detecting variants in the majority of the sea urchin species. Thus, it is possible that the low number of unique sequences in *D. antillarum* and *D. mexicanum* is a true reflection of the fact that in some sea urchin species only a few distinct SURL elements are present. Number of copies of TE elements per genome can vary greatly between species. For in-

stance, *mariner* elements are present in 17,000 copies in the horn fly *Haematobia irritans* but in only 3 copies in *Drosophila ananassae* (Robertson and Lampe 1995). The propagation of *P* elements is more difficult in the genome of *Drosophila simulans* than in that of *Drosophila melanogaster* (Kimura and Kidwell 1994). Alternatively, the low number of observed distinct elements in *Diadema* may mean that in this genus, SURL families are so different from each other that even highly degenerate primers can amplify only a single family. Britten et al. (1995), by using new primers designed for amplifying tunicate Ty3/*gypsy* elements, were able to identify SURLs of *Strongylocentrotus purpuratus* that were 57% different from the ones previously known from this species. Both interpretations (i.e., low number of elements and high sequence dissimilarities between elements) raise the possibility that these retrotransposons are subject to fairly high rates of extinction in their host genomes.

Phylogenetic Topologies of Retrotransposons and Hosts

Figure 1 presents the phylogenetic relationships, based on various kinds of data, of the echinoid species from which SURL elements were successfully amplified and sequenced. The split between the Cidaroidea (see table 1 for classification) and all other modern echinoids occurred no later than the Triassic. The Echinothuroida, represented in our phylogeny by *Araeosoma,* are probably an outgroup of all other Euechinoidea (Littlewood and Smith 1995), but their position is not well resolved, so we show them as part of a polytomy. Their fossil record goes back to 100 MYA (Smith 1984), which is a minimum estimate of the time they have been isolated from Euechinoidea. The Pedinoida, represented by *Caenopedina,* have a fossil record going back to the Upper Triassic (Smith 1984), approximately 200 MYA, and are definitely nested within the Euechinoidea, but their relationship to the other orders is unclear. Smith (1984) shows them as ancestral to the Diadematoida, while Littlewood and Smith (1995), based on morphological analysis of a single fossil genus, consider them an outgroup of the lineage leading to the Irregularia. We show their phylogenetic position as a part of a polytomy but consider the split from the Echinacea as having occurred at least 200 MYA. The rest of the phylogenetic relations are based on solid evidence, even if the time of splitting between some genera may be unknown. In many cases, the divergence times are based on substantial stratigraphic and molecular evidence, thus providing useful guideposts for determining rates of retrotransposon divergence. Many of the species we used are found on either side of the Isthmus of Panama, the geologically best dated vicariant event separating marine organisms (Coates and Obando 1996). Given the uncertainties of the fossil record and the difficulties of obtaining dates of divergence under the assumption that rates of molecular divergence are constant, the dates of splitting in figure 1 must be considered as having large errors. However, divergence dates within the correct order of magnitude are adequate for this study.

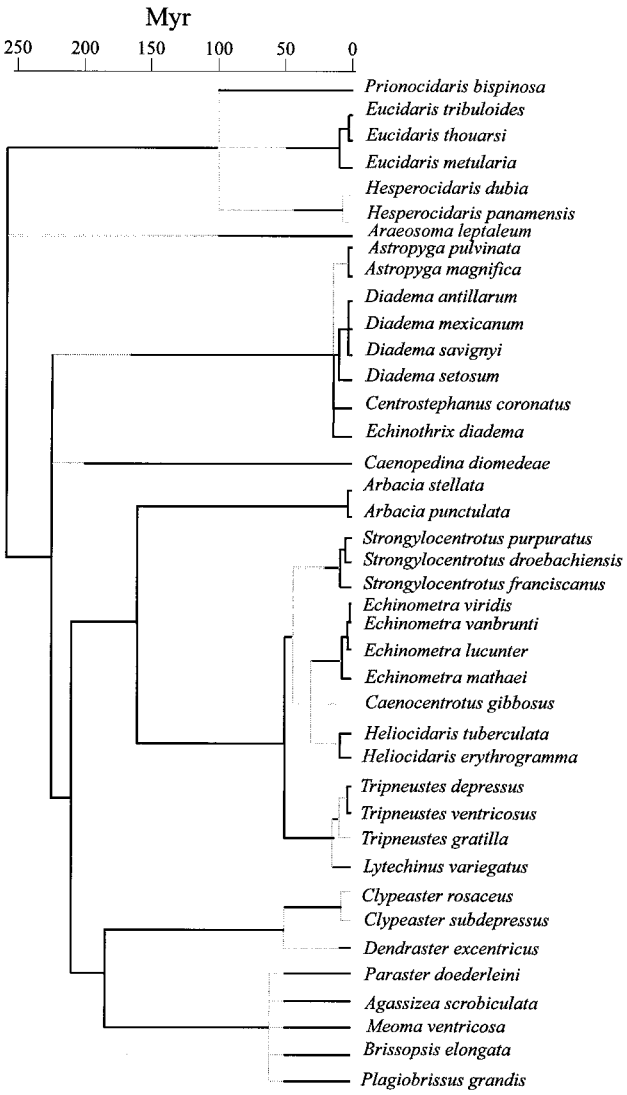

FIG. 1.—Phylogenetic relations and time of splitting between species from which SURL elements were successfully amplified and sequenced. Branches in which the time of splitting is supported by molecular, biogeographic, or fossil evidence are joined by heavy black lines. Branches for which there is no information on time since divergence are light gray. Phylogenetic uncertainties are shown as polytomies. The known stratigraphic range of each taxon, taken from Moore (1966) for genera and from Smith (1984) for higher categories, is also shown by heavy black lines along each branch. Species listed by Chesher (1972) as having been separated by the Isthmus of Panama are shown as having split 3 MYA, despite twofold variation in the degree of mitochondrial DNA divergence (Lessios 1998). Phylogeny and times of splitting in the genera *Diadema, Centrostephanus, Echinothrix,* and *Strongylocentrotus* are based on 1,302 bp of the Cytochrome Oxidase I (COI) and ATPase 6 and 8 mitochondrial regions (*Strongylocentrotus* from Kessing 1991; other species from unpublished data), of *Echinometra* on 580–640 bp of the COI region (Palumbi 1996; Lessios 1998), and of *Eucidaris* from 640 nt of the COI region (Lessios et al. 1999). The time of splitting between the two species of *Heliocidaris* is the midpoint between the range defined by restriction fragment length polymorphism comparisons of mitochondrial DNA (McMillan, Raff, and Palumbi 1992) and single- copy genomic DNA divergence (Smith, Boom, and Raff 1990). Phylogeny of higher categories is from Littlewood and Smith (1995), and times of divergence are from Smith (1989), Smith, Lafay, and Christen (1992), and Smith and Littlewood (1994).

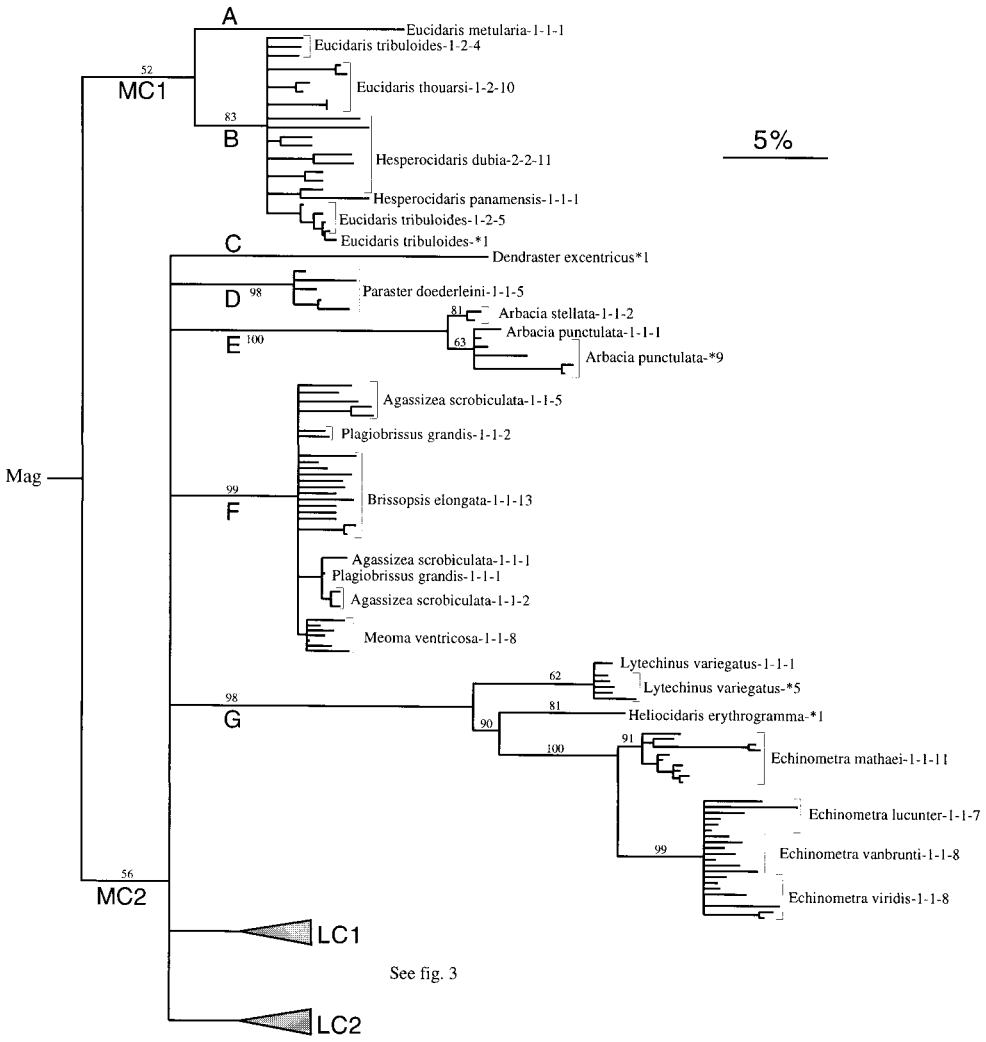

FIG. 2.—Part of the neighbor-joining (Saitou and Nei 1987) phylogenetic tree based on 263-bp sequences from the RT region of sea urchin SURLs. Numbers next to branches indicate support from bootstrapping the tree in 1,000 iterations. Branches with less than 50% support have been collapsed. Bootstrap values for nodes close to terminal clades have been omitted to maintain figure clarity. Letter codes next to branches indicate clades discussed in the text. Details of clades LC1 and LC2 are shown in figure 3. Sequences obtained by Springer et al. (1995) are identified by asterisks. Identical sequences from different clones are treated as single retrotransposable elements. Each element (or polytomy of elements) is identified by the name of the species from which it was amplified, followed by the number of sea urchin individuals in which it was found, followed by the number of independent amplifications that produced it, followed by the number of clones from which it was obtained. When the same sequence was obtained from two different species, the names of the species are joined with an ampersand.

Figures 2 and 3 show a neighbor-joining (Saitou and Nei 1987) tree based on Kimura-corrected distances among these sequences and rooted on a homologous sequence of RT of the *mag* element of *Bombyx mori* (Michaille et al. 1990). Springer and Britten (1993) have found that the *mag* element is the closest outgroup of SURLs in the *gypsy* family. Branches with less than 50% bootstrap support have been collapsed.

The SURL phylogenetic tree shows little overall resemblance to the host tree, as would be expected if subfamilies or retrotransposons began diverging prior to the speciation events in sea urchins. However, each clade is not a cluster of random associations between retrotransposons. Instead, the phylogenetic signature of the host is in most cases clearly evident within the subfamilies represented by each clade. The outgroup to all other SURLs is clade MC1, which includes elements from only the Cidaroidea. The average distance between clades MC1 and MC2 is approximately 34%, which, following Britten et al.'s (1995) retrotransposon classification by degree of divergence, would qualify them as different families. Given that cidaroids are the outgroup of all other echinoids, comparisons between these two major clades could be considered as orthologous. However, some elements of *H. panamensis* and all of the elements of *Prionocidaris bispinosa*, both members of the subclass Cidaroidea, are found in MC2. Thus, two interpretations are possible: the duplication of elements that lead to these two families could predate (by a large margin) the split of the two echinoid subclasses, or the high similarity of the elements from *Prionocidaris* and *Hesperocidaris* to those of euechi-

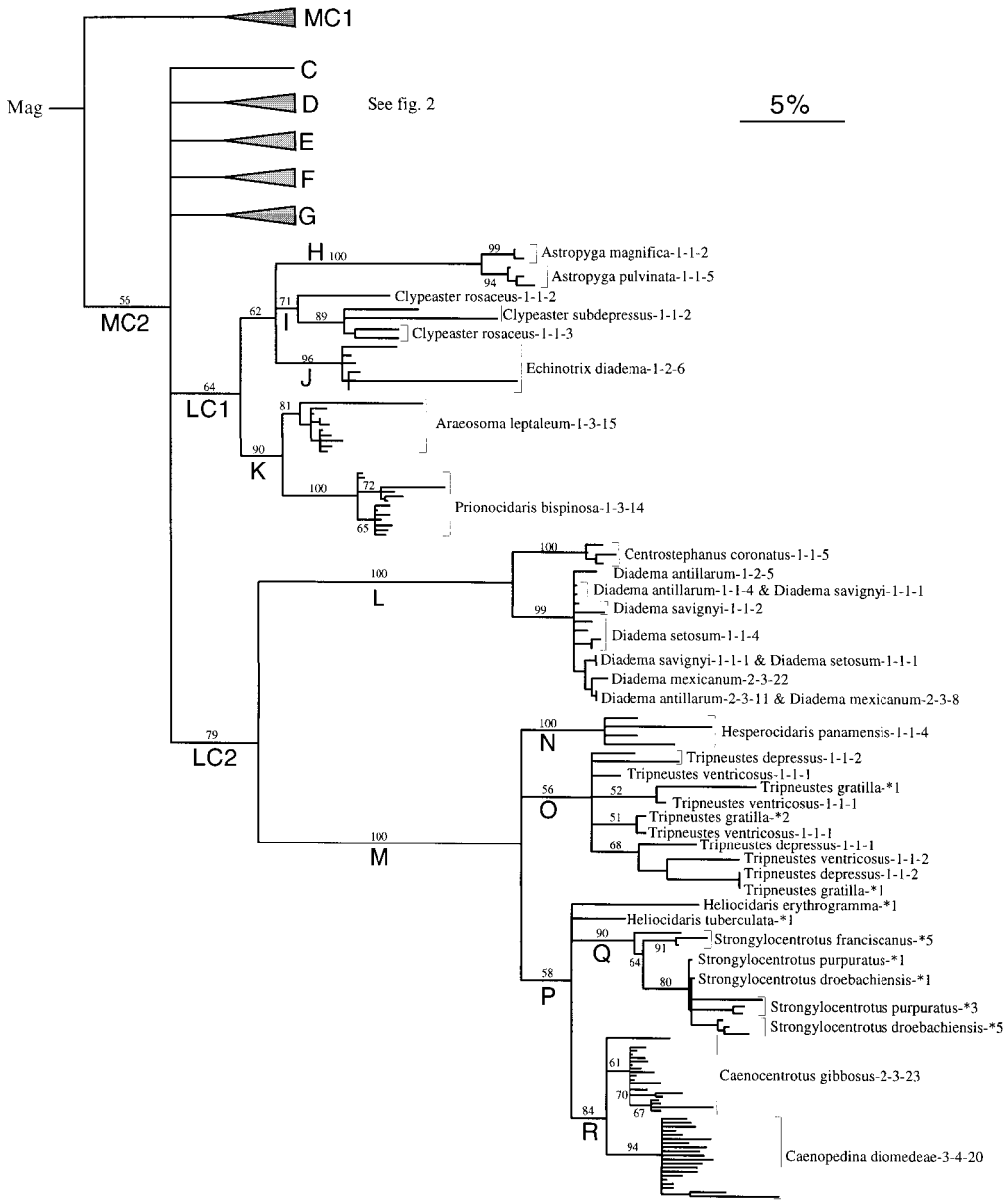

FIG. 3.—Second part of the neighbor-joining (Saitou and Nei 1987) phylogenetic tree based on 263-bp sequences from the RT regions of sea urchin SURLs, showing details of clades LC1 and LC2. All conventions and explanations are the same as in figure 2, in which details of clades MC1, C, D, E, F, and G are shown.

noids can be considered indicative of a horizontal transmission (see below).

Within clade MC1, the single element of *Eucidaris metularia* we were able to amplify forms its own clade,clade A, sister to clade B, which is composed of elements of the other two species of *Eucidaris,* but also of two species of *Hesperocidaris.* The average Kimura distance between clades A and B is 15.9%. Thus, each of these clades is a separate subfamily resulting from duplications that preceded the split between the host species.

Major clade MC2 is composed of seven clades with unresolved relationships. Clades C and D are each composed of elements from a single species. Clade E contains all elements of *Arbacia,* with those of the two species, presumably separated for at least 3 Myr by the Isthmus of Panama (Chesher 1972), segregating in separate subclades. Clade F is composed entirely of elements from spatangoids and contains all elements from this echinoid order except of those from *Paraster.* Clade G contains clear evidence of vertical transmission of retrotransposons and coevolution with their hosts. Elements of the three closely related Neotropical species of *Echinometra* cluster together. Unlike the case of *Arbacia,* there is no clearly resolved separation between elements from species on the two sides of the Isthmus of Panama, but in a short stretch of DNA such as the one we sequenced, such unresolved relationships in the terminal branches may be expected. Elements from the Indo-West Pacific *Echinometra mathaei,*

which is more distantly related to Neotropical species of *Echinometra* (Palumbi 1996), form a sister clade. A single element of the echinometrid *Heliocidaris erythrogramma* is sister to those from the *Echinometra*s, and elements from the toxopneustid *Lytechinus,* separated from the echinometrids for 60 Myr, form the outgroup of the echinometrid elements. Thus, in clade G, the phylogeny of TEs duplicates the phylogeny of the hosts, and on phylogenetic grounds, it is justifiable to consider this lineage a single subfamily of SURL retrotransposons.

The next clade, LC1 (fig. 3), cannot be reconciled with the host phylogeny, and must, therefore, contain paralogous comparisons and/or horizontal transfers. That elements from *Araeosoma* and *Prionocidaris* (clade K) form a sister clade to those of *Astropyga, Echinothrix,* and *Clypeaster* does not create a problem as far as the reconciliation of host and transposon tree topologies is concerned. However, the elements of *Clypeaster* (clade I), a sand dollar separated from the Diadematoida for 200 Myr, are nested in the subclade composed of *Astropyga* (H) and *Echinothrix* (J). Either there has been a horizontal transfer of SURLs between *Clypeaster* and a diadematid sea urchin, or each of clades H, I, and J represent a separate SURL family duplicated in the genome of the common ancestor of Diadematoida and Echinacea. In contrast to the case of *Echinometra,* and in agreement with the case of *Arbacia,* elements from the two species of *Astropyga* from the two sides of Central America (clade H) cluster separately.

The topology of clade LC2 is also difficult to reconcile with host phylogeny, unless paralogous comparisons and horizontal transfers are invoked. Subclade L is clearly a family of retrotransposons that amplified only in the diadematids *Diadema* and *Centrostephanus,* with elements from each genus clustering separately. The relations of elements from *Diadema* within this clade do not follow the phylogeny of the species of this genus, but this is most likely the result of lack of phylogenetic resolution, rather than the existence of different subfamilies. Subclade M, on the other hand, is composed of elements of which the phylogeny does not always bear a relation to that of the hosts. That some elements of the cidaroid *H. panamensis* are in a subclade (N) separate from the rest of the subclades is, of course not inconsistent with sea urchin phylogeny. That another subclade (O) is composed entirely of elements from the toxopneustid *Tripneustes* is also compatible with host phylogeny. However, within subclade O, the relations between elements from the three species do not cluster according to their source species. One of our *Tripneustes depressus* sequences is identical to one of Springer et al.'s (1995) sequences from *T. gratilla* (except for a 1-codon deletion in the latter), and thus the two elements cluster together. Two other branches suggest higher similarities between elements from the Indo-West Pacific *T. gratilla* and the Caribbean *Tripneustes ventricosus* than between elements from a single species. However, the degree of bootstrap support for these two clusters is only marginally higher than 50%, so it is doubtful that they reflect true phylogenetic relationships. Despite the different ways in which this study and that of Springer et al. (1995) treated the data, the tree we obtained for elements of *Strongylocentrotus* (subclade Q) is the same as theirs. We agree with them that paraphyletic elements in *S. purpuratus* are the result of slightly earlier dates of divergence between the elements than the split of this species from *Strongylocentrotus droebachiensis.* Finally, clade R cannot be reconciled with vertical transmission of SURLs without postulating an extreme slowdown in the rate of their evolution, because it includes all elements from *Caenocentrotus* and *Caenopedina,* members of two sea urchin orders that diverged at least 200 MYA, and is likely to be the product of horizontal transmission (see below).

Integrity of the Coding Region

Forty-six of the 315 sequences included in this study (14.6%) had an interruption of the RT ORF, consisting of insertions of up to 5 nt, deletions of up to 22 nt, and stop codons, with a high frequency of more than one type of interruption. In all but three cases, these mutations definitely convert these elements to inactive copies. Obviously, this is an underestimate of the total number of inactive copies, as other elements may have interruptions outside the sequence we analyzed. The high proportion of inactive elements is consistent with the view that in the short term SURLs evolve as pseudogenes (Springer et al. 1995). In seven cases, two or more elements share the same indel or stop codon, suggesting that they are all derived from the same ancestral inactive element and that differences in their sequences were acquired during vertical transmission in the genome of their host.

An interesting case of ORF interruption is a 12-bp deletion present in elements from two different species, *T. depressus* and *T. ventricosus.* Two of the three clones with this deletion also had stop codons, indicating that these elements are indeed inactive. As *T. depressus* and *T. ventricosus* were separated at least 3 MYA as the result of the completion of the Isthmus of Panama (Chesher 1972), this deletion has been present in the genome of *Tripneustes* for at least this period of time. All elements of *Astropyga magnifica* and *Astropyga pulvinata* are also inactive. These two species are also assumed to have been isolated by the Isthmus of Panama (Chesher 1972). However, unlike the elements of *Tripneustes,* those of *Astropyga* do not share the same deleterious frameshifts, so their inactivation may be due to independent events more recent than the separation of the two species.

In addition to those in *Astropyga,* 9 of 10 elements in *Araeosoma leptaleum,* 4 of 5 elements in *H. panamensis,* and, as noted by Springer et al. (1995), 3 of 4 elements in *S. purpuratus* and all 3 elements in *Strongylocentrotus franciscanus* had interrupted ORFs. Although other explanations can be offered, the predominance of inactive elements among those amplified from some species could indicate that some SURL families may no longer be present as active copies in the host genome. This type of inactivation and subsequent sto-

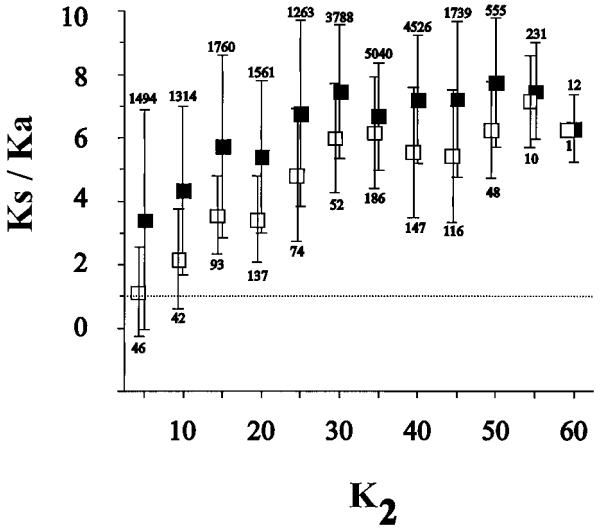

FIG. 4.—Average ratio ± one standard deviation of synonymous (K_s) to nonsynonymous (K_a) substitutions for each five-point interval of Kimura (1980) two-parameter percentage of nucleotide difference (K_2). Filled squares represent averages of all comparisons, and open squares represent averages of elements known to be inactive because of open reading frame interruptions in the sequenced region. Numbers above or below the bars indicate the number of comparisons from which the means and standard deviations were calculated. Calculation of synonymous and nonsynonymous substitutions is based on equations of Pamilo and Bianchi (1993) and Li (1993). The dotted line marks the 1:1 ratio that would be expected if changes were equally likely at all sites.

chastic loss may explain discontinuities of TE subfamilies in closely related host species. Lohe et al. (1995) concluded that the predominant mode of evolution of *mariner*-like elements is vertical inactivation and stochastic loss balanced by invasions into new lineages through horizontal transmission.

Synonymous Versus Nonsynonymous Substitutions

Figure 4 shows the ratio of synonymous (K_s) to nonsynonymous (K_a) substitutions between all elements and between the 46 elements known to be incapable of their own retrotransposition because of ORF interruptions, plotted against the values of Kimura-corrected overall percent divergence (K_2). In addition to including the 46 known inactive elements, the all-elements category undoubtedly includes additional SURLs that are inactive because of indels or stop codons outside the sequenced area. However, as it also contains active retrotransposons, it provides an interesting contrast to the inactive elements. Because there are 27,264 pairwise comparisons, the presentation of the data has been simplified by averaging the ratios for each five-point interval of K_2. Of these comparisons, 3,933 have been excluded because they yielded corrected K_s values that were undefined or K_a values equal to zero. Forty-seven additional comparisons involved unreasonably high K_s values (1,255%–1,502%) and were excluded in order to avoid biasing the means.

In agreement with what was noted earlier by Springer et al. (1995), few comparisons between SURL elements have K_s/K_a ratios that are less than or equal to one, demonstrating the influence of purifying selection in the evolution of these retrotransposons. Springer et al. (1995) noticed an increase in the K_s/K_a ratio with increasing overall divergence, a trend that was also found by McAllister and Werren (1997) in the Nasonia Transposable Element (another retrotransposon of the Ty3/*gypsy* group), and by Lathe et al. (1995) in the non-LTR element R1. This trend is also present in our data for K_2 values lower than 30%, but the average ratios appear to level off with higher total divergence, probably as the result of saturation of the synonymous sites due to multiple hits. Interestingly, the comparisons between elements known to be inactive shows the same trend as the mixture of active and inactive elements, but the average K_s/K_a ratios start at 1 and remain lower for any given value of divergence. Unfortunately, because of the nonindependence of the pairwise comparisons, no statistical tests can be performed to verify that this difference is significant.

Springer et al. (1995) suggested two possible explanations for the increasing excess of silent substitutions with time since divergence. One is that some nonsynonymous substitutions are under weak selection; K_a values initially increase at a high rate until these sites are saturated, but then they level off. The other possible explanation is that transposable elements evolve in the short term as pseudogenes, but in the long term they bear the mark of strong selection for RT activity that has acted during retrotransposition events. A third possible explanation is that the apparent trend is an artifact of the PCR technique used to detect the elements. Primers are designed for conserved sites of the RT coding regions. Any element that has been evolving as a pseudogene for a long time may become a less competitive target for PCR amplification than those with a more recent active history. Thus, distant comparisons would involve a higher proportion of elements that either are still active or have been recently inactivated. Ultimately, of course, the distinction between the second and the third explanations is mostly semantic in that, except through deletions, retrotransposon DNA is not excised from the host genome; it only becomes more difficult to recognize as such.

Whatever the reason for the increasing proportion of synonymous to nonsynonymous substitutions with overall divergence, the high ratios indicate that SURLs transpose frequently enough to be subject to purifying selection. However, that inactive elements have lower K_s/K_a ratios indicates that the inactivations are sufficiently old for nonsynonymous sites to have accumulated mutations that visibly affect their K_s/K_a ratio relative to that of a mixture of active and inactive elements. This remains true even in comparisons between distantly related SURLs, in which one might have expected that the long active history would have diluted the mark of recent unconstrained evolution. Unlike active elements, in the short term ($K_2 < 5\%$), the inactive elements evolve as true pseudogenes with average K_s/K_a ratios close to 1.

Table 2
Absolute and per-Myr Divergence for All Sites (K_2), Synonymous Sites (K_s), and Nonsynonymous Sites (K_a) Between SURL Elements from Taxa in Which (a) Element Phylogeny Mirrors Host Phylogeny and Dates of Divergence Between Host Taxa are Established by Independent Evidence and (b) the Element Phylogeny Relative to that of the Host Suggests the Possibility of Horizontal Transfer

		Divergence					Rate		
Clade[a]		Time[b] (Myr)	K_2[c] (%)	K_s[d] (%)	K_a[d] (%)	K_s/K_a	K_2[c] (%/Myr)	K_s[d] (%/Myr)	K_a[d] (%/Myr)
	(a) Known dates of host divergence								
G	*Echinometra lucunter* vs. *Echinometra viridis*	2	3.31	4.26	2.13	2.00	1.66	2.13	1.07
B	*Eucidaris tribuloides* vs. *Eucidaris thouarsi*	3	3.52	7.03	1.86	4.24	1.17	2.34	0.62
E	*Arbacia punctulata* vs. *Arbacia stellata*	3	4.83	8.60	3.06	2.81	1.61	2.87	1.02
G	*Echinometra vanbrunti* vs. *E. lucunter* and *E. viridis*	3	3.39	5.96	2.35	2.54	1.13	1.99	0.78
H	*Astropyga magnifica* vs. *Astropyga pulvinata*	3	3.63	4.93	3.17	1.56	1.21	1.64	1.06
L	*Diadema antillarum* vs. *Diadema savignyi*	3	1.38	1.88	1.05	1.79	0.46	0.63	0.35
L	*Diadema mexicanum* vs. *D. antillarum*	3	1.20	1.98	0.60	3.30	0.40	0.66	0.20
L	*Diadema mexicanum* vs. *D. savignyi*	3	1.93	3.30	1.12	2.95	0.64	1.10	0.37
O	*Tripneustes ventricosus* vs. *Tripneustes depressus*	3	7.64	21.63	5.40	4.01	2.55	7.21	1.80
Q	*Strongylocentrotus drebachiensis* vs. *Strongylocentrotus purpuratus*	3	3.34	1.78	3.54	0.50	1.11	0.59	1.18
G	*Echinometra mathaei* vs. *E. vanbrunti* and *E. lucunter* and *E. viridis*	6	9.18	21.48	4.09	5.25	1.53	3.58	0.68
L	*Diadema setosum* vs. *D. savignyi* and *D. antillarum* and *D. mexicanum*	9	1.71	2.72	1.18	2.31	0.19	0.30	0.13
L	*Centrostephanus coronatus* vs. *Diadema*	15	8.24	16.38	4.35	3.77	0.55	1.09	0.29
Q	*Strongylocentrotus franciscanus* vs. *S. purpuratus* and *S. drebachiensis*	18	6.30	6.98	4.88	1.43	0.35	0.39	0.27
G	*Lytechinus variegatus* vs. *Heliocidaris*[e] and *Echinometra*	60	18.45	44.19	10.16	4.35	0.31	0.74	0.17
MC1 vs. MC2	Cidaroidea[e] vs. Euechinoidea[e]	260	33.87	126.65	16.75	7.56	0.13	0.49	0.06
	(b) Suspected horizontal transfers								
K	*Araeosoma leptaleum* vs. *Prionocidaris bispinosa*	230	7.43	11.55	5.05	2.29	0.03	0.05	0.02
N	*Caenocentrotus gibbosus* vs. *Caenopedina diomedeae*	230	6.27	12.11	3.71	3.26	0.03	0.05	0.02
LC1 ...	*Clypeaster* vs. *Astropyga*	210	17.26	18.34	15.15	1.21	0.08	0.09	0.07
LC1 ...	*Clypeaster* vs. *Echinothrix*	210	13.60	24.6	8.3	2.96	0.06	0.12	0.04
M	*Hesperocidaris panamensis*[e] vs. *Tripneustes* and *Heliocidars*[e] and *Strongylocentrotus* and *Caenocentrotus* and *Caenopedina*	230	17.32	37.59	8.86	4.24	0.08	0.16	0.04

[a] See figures 2 and 3 for clade designations.
[b] See legend of figure 1 for references.
[c] Corrected according to Kimura (1980).
[d] Corrected according to Pamilo and Bianchi (1993) and Li (1993).
[e] Only elements from this taxon that belong to the clade shown on the left are included in the comparison.

Rates of Evolution

A problem in the study of evolution of transposable elements is that many of the conclusions are based on assumptions regarding their rates of substitution. To calculate such rates, good estimates of divergence times are needed. The major advantage of studying retrotransposons in echinoids, particularly in species separated by the Isthmus of Panama, is that their well-studied phylogeny affords fairly accurate dates of separation between host species and, thus, minimum time (barring horizontal transmission) that the TEs have also been evolving independently. Rates of evolution in SURL elements were estimated by comparing sequences from pairs of echinoid taxa for which the retrotransposon phylogeny is concordant with the host phylogeny. To minimize the effect of comparison between paralogous elements, only elements from the same clade were compared, and only if independent evidence existed for the divergence times of the hosts (see fig. 1). This does not guarantee that all comparisons are orthologous, but it does provide an estimate of the maximum rate of evolution for these elements. Table 2 shows rates of syn-

onymous, nonsynonymous, and total substitutions between the elements from pairs of species that fulfill these conditions. The results indicate that the rate of overall divergence of retrotransposons (K_2) can vary between 0.13% and 2.55% per Myr with a mean of 0.94%/Myr. Comparisons involving *Diadema*, which has a low detected distinct copy number (see above) and may include horizontal transfer of elements between species (see below), tend to have the lowest values. *Tripneustes*, in which most of the elements may have been inactive in the last 3 Myr, has the highest value. Our estimates of the overall rate of divergence of SURLs tend to be slightly higher but are in fairly good agreement with those of Springer et al. (1995), particularly if one considers that the estimates of divergence times are only approximate.

A more worrisome point of disagreement between the estimates of Springer et al. (1995) and those of our analysis is the discrepancy between rates of evolution in synonymous or nonsynonymous sites. For example, they calculate the K_s value for the comparison of *S. purpuratus* with *S. droebachiensis* as 3.6% and that between these two species and *S. franciscanus* as 18.1%, whereas our equivalent values are less than half of theirs (table 2). The differences are caused, to a very small degree, from our analysis of a slightly shorter sequence and from our performing the comparisons between elements rather than between clones and, to a large degree, from different methods of estimating these parameters. Springer et al. (1995) used the method of Li, Wu, and Luo (1985), whereas we used that of Pamilo and Bianchi (1993) and Li (1993). The Li, Wu, and Luo (1985) method counts twofold-degenerate sites as one third synonymous and two thirds nonsynonymous, which tends to overestimate the rate of synonymous substitution at such sites in closely related sequences (Li 1997, p. 89). Based on our estimates from reanalysis of the Springer et al. (1995) data and the additions of the species we assayed, the average rate of synonymous substitutions in SURLs is 1.73% per Myr, which still upholds the conclusion of Springer et al. (1995) that it is similar to the rate of divergence of single-copy nuclear DNA (1.1%–1.5% per Myr).

The conclusion of Springer et al. (1995) that K_s/K_a ratios increase with time since divergence is borne out by our analysis of all pairwise comparisons (fig. 4), but is not as evident in table 2. Species that have been separated for approximately 3 Myr have ratios that vary between 0.5 and 4.2, which points to the largely stochastic nature of substitutions and the high variance in comparisons of elements separated for fairly short periods.

Horizontal Transmission

Various lines of evidence have been presented at various times to support horizontal transmission in transposable elements. Those based on the assumption of higher rates of evolution in retrotransposons than in the rest of the genome or on the apparent absence of TEs from phylogenetically intermediate host species have been criticized (review in Cummings 1994). Cases in which elements from distantly related host species show an extremely high degree of similarity, however, are hard to explain as anything but the result of a horizontal transfer. To be sure, it is always possible to make ad hoc hypotheses of unusual evolutionary slowdowns in TE evolution and reconcile their phylogeny with that of the host by postulating that only comparisons between the most closely related elements are orthologous, while all others are paralogous. However, there are cases in which good agreement of the phylogenies at higher levels make the assumption of horizontal transfer the most parsimonious explanation.

As has been obvious from our discussion of the SURL phylogeny, we made vertical transmission our null hypothesis and thus adapted as an operational definition of a SURL subfamily the most distal clade in which the phylogenetic relations of the retrotransposons match the phylogenetic relations of the host. However, had this definition been strictly applied, only comparisons of elements between *Caenocentrotus* and *Caenopedina* (clade K in fig. 3) and those between *Araeosoma* and *Prionocidaris* (clade R) would have been deemed orthologous. In this case, the calculated rate of retrotransposon evolution would have been 0.03%–0.08%/Myr (table 2). Although we cannot categorically state that evolutionary rate variation of this magnitude is impossible, the corollary assumption that the entire SURL phylogeny is the result of paralogous comparisons would lead to the conclusion that all agreement between it and the sea urchin phylogeny is the result of chance. It seems much more reasonable to suggest that there was a horizontal transfer of SURLs from the lineage leading to *Araeosoma* to that leading to *Prionocidaris* (clade K), between *Caenocentrotus* and *Caenopedina* (clade R), between *Clypeaster* and the common diadematoid ancestor of *Astropyga* and *Echinothrix* (clades H–J), and between a camarodont ancestor and the lineage leading to *Hesperocidaris* (clade M) (see table 2 and fig. 3). Note that in the case of *H. panamensis*, both the elements that have been presumably transferred horizontally and those that pertain to the original cidaroid SURL lineage are present in the same individual.

In addition to the evidence for horizontal transfer arising from SURL phylogeny and evolutionary rates, there is evidence in our data of more recent horizontal transfers. This consists of elements that are found in different species but are indistinguishable in the RT region we sequenced. We synonymized individual sequences that were found in clones from the same species, but there are four cases of identical sequences that belong to elements from different species (table 3). It is highly unlikely that these identical sequences are the result of PCR contamination, because the sequence from *T. gratilla* comes from Springer et al. (1995), while that from *T. depressus* comes from our laboratory, which has not worked with DNA from *T. gratilla.* The identity of sequences between *D. savignyi* and *D. setosum* need not be the result of horizontal retrotransposition, because these two species occasionally hybridize (Lessios and Pearse 1996), but the other three cases involve species that are allopatric. There is an astronomically low prob-

Table 3
Elements from Different Sea Urchin Species with Identical RT Sequences

	Time (Myr)	Rate of divergence[a] (%/Myr)	p[b]
Diadema antillarum and *Diadema mexicanum*	3	0.40	3×10^{-151}
D. antillarum and *Diadema savignyi*	3	0.46	3×10^{-151}
D. savignyi and *Diadema setosum*	9	0.19	5×10^{-150}
Tripneustes depressus and *Tripneustes gratilla*[c]	~5[d]	1.57	1×10^{-150}

NOTE.—Elements with identical sequences from the above species are designated in figure 3 with labels composed of names of both species connected with an ampersand.

[a] Average Kimura (1980) two-parameter distance of all SURL elements from the same two sea urchin species, divided by time.

[b] Probability of identity by descent calculated for a 250-bp sequence evolving at the rate and for the length of time indicated, under the assumption that all substitutions at each site are equally likely, using the formulas found in Li (1997, p. 70).

[c] Elements are identical except for a single codon deletion in *T. gratilla.*

[d] Time of separation is unknown. Even if it is assumed to be 2 Myr, the probability of identity by descent is still vanishingly low.

ability that two 250-bp sequences separated for the same time as their hosts will be identical by descent if they evolve at the average rate of other SURL elements from the same sea urchin species (table 3). We therefore see no explanation for three of the four cases of RT sequence identity between elements from different species other than horizontal transfer. Unlike the possible horizontal transfers suggested by the SURL phylogeny, these transfers must be quite recent.

One of the problems in dealing with horizontal transmission of TEs is that very little is known about the actual mechanisms through which this transmission may occur, even in insects, for which evidence exists that parasites (Houck et al. 1991) and viruses (Friesen and Nissen 1990) can act as vectors. Some retrotransposons have an ORF in the same position as the *env* gene in retroviruses, which codes for a retroviral-like envelope protein (Springer and Britten 1993; Tanda, Mullor, and Corces 1994; Kim et al. 1994). These elements are potentially infectious, but so little is known about parasites of marine invertebrates and potential vectors of SURLs that it is pointless to speculate on how they may have been transmitted between species. Nevertheless, the question of how retrotransposons could be physically transferred between sea urchins with nonoverlapping geographical ranges needs to be addressed. Transfers identified by their phylogenetic signatures, such as those that involve *Araeosoma, Prionocidaris, Hesperocidaris, Caenocentrotus,* and *Clypeaster,* may have happened in ancestors of the modern species with ranges that coincided at a time when ocean conformation and currents were different. However, the proposed horizontal transfers of elements with identical RT sequences must have occurred after the modern sea urchin species had evolved. *Diadema savignyi, D. setosum,* and *T. gratilla* are Indo-West Pacific species, and *D. mexicanum* and *T. depressus* are limited to the eastern Pacific, while *D. antillarum* is found on both sides of the Atlantic. It is definitely known that larvae of *D. savignyi* occasionally settle on islands of the eastern Pacific (Lessios et al. 1996), so the same possibility must be considered for contact between *T. gratilla* and *T. depressus.* But how can the transfer of transposable elements between *D. mexicanum* and *D. antillarum* and between *D. savignyi* and *D. antillarum* be explained biogeographically? The only explanation we can offer is that the vector may have traveled either in ship ballast water through the Panama Canal (Carlton and Geller 1993) or on the currents that go around the southern tip of Africa.

Even though we cannot explain how the transfers may have occurred, we think that the evidence for horizontal transfer in our data suggests that SURLs do occasionally transfer horizontally. Previous conclusions to the contrary (Springer et al. 1995) are the result of the relative rarity of such transfers, which makes it necessary that many species be sampled in order to detect them.

Conclusions

The results presented here show that although more than one subfamily of SURLs may occasionally occur in the same genome, as a rule clones from the same host species are very similar. In some host species, the numbers of distinct elements may be low. More than 15% of these copies may be inactivated with deleterious frameshifts or stop codons, but the RT region of these elements bears obvious marks of purifying selection. Comparison of distant elements shows a higher ratio of nonsynonymous to synonymous substitution, suggesting that only retrotransposons with conserved expression of RT survive for a long time. The rate of synonymous substitution in this region is comparable with the rate of substitution of sea urchin single-copy DNA. Elements are most frequently transferred vertically, and most of their phylogeny reflects host cladogenesis and retrotransposon duplication. However, contrary to previous findings from smaller samples of host species, there is also evidence of retrotransposon horizontal transmission.

Acknowledgments

We thank M. S. Springer for providing SURL sequences used in the Springer et al. (1995) study, C. Luna for help in the laboratory, B. D. Kessing for extensive data analysis and advice, and D. L. Swofford for permission to publish results analyzed with a test version of PAUP*. P. Barnes, G. Paulay, and J. S. Pearse collected specimens from the Indo-Pacific. We thank S.

Pomponi, P. W. Glynn, and G. M. Wellington for invitations to join cruises on oceanographic vessels in which many of the sea urchin specimens were collected, and we thank R. Mooi for identification of echinoid specimens. B. D. Kessing, M. A. McCartney, and K. Ziegler commented on the manuscript. This work was supported by funds from the Smithsonian Institution Molecular Evolution Program and a Smithsonian postdoctoral fellowship to P.G.

LITERATURE CITED

ALBEROLA, T. M., and R. DE FRUTOS. 1993*a*. Distribution of *gypsy* sequences in *Drosophila* species of the obscura subgroup. Hereditas **118**:131–137.

———. 1993*b*. *Gypsy* homologous sequences in *Drosophila subobscura* (*gypsyDS*). J. Mol. Evol. **36**:127–135.

BRITTEN, R. 1996. Cases of ancient mobile element DNA insertions that now affect gene regulation. Mol. Phylogenet. Evol. **5**:13–17.

———. 1997. Mobile elements inserted in the distant past have taken on important functions. Gene **205**:177–182.

BRITTEN, R. J., T. J. MCCORMACK, T. L. MEARS, and E. H. DAVIDSON. 1995. *Gypsy*/Ty3-class retrotransposons integrated in the DNA of herring, tunicate and echinoderms. J. Mol. Evol. **40**:13–24.

BUCHETON, A. 1995. The relationship between the *flamenco* gene and *gypsy* in *Drosophila*: how to tame a retrovirus. Trends Genet. **11**:349–353.

CAPY, P., D. ANXOLABÉHÈRE, and T. LANGIN. 1994. The strange phylogenies of transposable elements: are horizontal transfers the only explanation? Trends Genet. **10**:7–12.

CARLTON, J. T., and J. B. GELLER. 1993. Ecological roulette: the global transport of nonindigenous marine organisms. Science **261**:78–82.

CHARLESWORTH, B., P. SNIEGOWSKI, and W. STEPHAN. 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. Nature **371**:215–220.

CHESHER, R. H. 1972. The status of knowledge of Panamanian echinoids, 1971, with comments on other echinoderms. Bull. Biol. Soc. Wash. **2**:139–158.

CLARK, J. B., P. C. KIM, and M. G. KIDWELL. 1998. Molecular evolution of *P* transposable elements in the genus *Drosophila*. III. The *Melanogaster* species group. Mol. Biol. Evol. **15**:746–755.

CLARK, J. B., W. P. MADDISON, and M. G. KIDWELL. 1994. Phylogenetic analysis supports horizontal transfer of *P* transposable elements. Mol. Biol. Evol. **11**:40–50.

COATES, A. G., and J. A. OBANDO. 1996. The geologic evolution of the Central American Isthmus. Pp. 21–56 *in* J. B. C. JACKSON, A. G. COATES, and A. BUDD, eds. Evolution and environment in tropical America. University of Chicago Press, Chicago.

COOK, J. M., and M. TRISTEM. 1997. 'SINEs of the times'—transposable elements as clade markers for their hosts. Trends Ecol. Evol. **12**:295–297.

CUMMINGS, M. P. 1994. Transmission patterns of eukaryotic transposable elements: arguments for and against horizontal transfer. Trends Ecol. Evol. **9**:141–145.

DANIELS, S. B., K. R. PETERSON, L. D. STAUSBAUGH, M. G. KIDWELL, and A. CHOVNICK. 1990. Evidence for horizontal transmission of the P transposable element between *Drosophila* species. Genetics **124**:349–355.

DOMBROSKI, B. A., S. L. MATHIAS, E. NANTHAKUMAR, A. F. SCOTT, and H. H. J. KAZAZIAN. 1991. Isolation of an active human transposable element. Science **254**:1805–1808.

DOOLITTLE, R. F., D. F. FENG, M. S. JOHNSON, and M. A. MCCLURE. 1989. Origins and evolutionary relationships of retroviruses. Q. Rev. Biol. **64**:1–29.

DURHAM, J. W. 1966. Evolution among the Echinoidea. Biol. Rev. Camb. Philos. Soc. **41**:368–391.

EANES, W., C. EWSLEY, J. H. HEY, D. HOULE, and J. AJIOKA. 1988. The fitness consequences of *P* element insertion in *Drosophila melanogaster*. Genet. Res. **52**:17–26.

EICKBUSH, D. G., W. C. LATHE III, M. P. FRANCINO, and T. H. EICKBUSH. 1995. R1 and R2 retrotransposable elements of *Drosophila* evolve at rates similar to that of nuclear genes. Genetics **139**:685–695.

EICKBUSH, T. H. 1994. Origin and evolutionary relationships of retroelements. Pp. 121–157 *in* S. S. MORSE, ed. Evolutionary biology of viruses. Raven Press, New York.

FINNEGAN, D. J. 1989. Eukaryotic transposable elements and genome evolution. Trends Genet. **5**:103–107.

FLAVELL, A. J. 1992. Ty1-copia group retrotransposons and the evolution of retroelements in the eukaryotes. Genetica **86**: 203–214.

FLAVELL, A. J., S. R. PEARCE, J. S. P. HESLOP-HARRISON, and A. KUMAR. 1997. The evolution of Ty1-*copia* group retrotransposons in eukaryote genomes. Genetica **100**:185–195.

FRIESEN, P. D., and M. S. NISSEN. 1990. Gene organization and transcription of TED, a lepidopteran retrotransposon integrated within the baculovirus genome. Mol. Cell. Biol. **10**: 3067–3077.

GARCIA-FERNÁNDEZ, J., G. MARFANY, J. BAGU, and E. SALO. 1993. Infiltration of mariner elements. Nature **364**:109–110.

GREEN, M. M. 1988. Mobile DNA elements and spontaneous gene mutation. Pp. 41–50 *in* M. E. LAMBERT, J. F. MCDONALD, and I. B. WEINSTEIN, eds. Eukaryotic transposable elements as mutagenic agents. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

HOUCK, M. A., J. B. CLARK, K. R. PETERSON, and M. G. KIDWELL. 1991. Possible horizontal transfer of *Drosophila* genes by the mite *Protolaelaps regalis.* Science **253**:1125–1129.

JENSEN, M. 1981. Morphology and classification of Euechinoidea Bronn, 1860: a cladistic analysis. Vidensk. Medd. Dan. Nathist. Foren. **143**:7–99.

KAZAZIAN, H. H., and J. V. MORAN. 1998. The impact of L1 retrotransposons on the human genome. Nat. Genet. **19**:19–24.

KESSING, B. D. 1991. Strongylocentrotid sea urchin mitochondrial DNA: phylogenetic relationships and patterns of molecular evolution. M.S. dissertation, University of Hawaii, Honolulu.

KIDWELL, M. G. 1983. Evolution of hybrid dysgenesis in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80**:1655–1659.

———. 1992. Horizontal transfer. Curr. Opin. Genet. Dev. **2**: 863–873.

———. 1993. Lateral transfer in natural populations of eukaryotes. Annu. Rev. Genet. **27**:235–256.

KIDWELL, M. G., and D. LISCH. 1997. Transposable elements as sources of variation in animals and plants. Proc. Natl. Acad. Sci. USA **94**:7704–7711.

KIM, A., C. TERZIAN, P. SANTAMARIA, A. PELISSON, N. PRUD'HOMME, and A. BUCHETON. 1994. Retroviruses in invertebrates: the *gypsy* retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **91**:1285–1289.

KIMURA, K., and M. G. KIDWELL. 1994. Differences in *P* element population dynamics between the sibling species *Drosophila melanogaster* and *Drosophila simulans.* Genet. Res. **63**:27–38.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**:111–120.

KONIECZNY, A., D. F. VOYTAS, M. P. CUMMINGS, and F. M. AUSUBEL. 1991. A superfamily of *Arabidopsis thaliana* retrotransposons. Genetics **127**:801–809.

KRISHNAPILLAI, V. 1996. Horizontal gene transfer. J. Genet. **75**: 219–232.

KUNZE, R., H. SAEDLER, and W. E. LÖNNIG. 1997. Plant transposable elements. Pp. 331–470 *in* J. A. CALLOW, ed. Advances in botanical research. Academic Press, San Diego.

LATHE, W. C., W. D. BURKE, D. G. EICKBUSH, and T. H. EICKBUSH. 1995. Evolutionary stability of the R1 retrotransposable element in the genus *Drosophila.* Mol. Biol. Evol. **12**: 1094–1105.

LATHE, W. C., and T. H. EICKBUSH. 1997. A single lineage of R2 retrotransposable elements is an active, evolutionarily stable component of the *Drosophila* rDNA locus. Mol. Biol. Evol. **14**:1232–1241.

LESSIOS, H. A. 1998. The first stage of speciation as seen in organisms separated by the Isthmus of Panama. Pp. 186–201 *in* D. J. HOWARD and S. BERLOCHER, eds. Endless forms: species and speciation. Oxford University Press, Oxford.

LESSIOS, H. A., B. D. KESSING, D. R. ROBERTSON, and G. PAULAY. 1999. Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. Evolution (in press).

LESSIOS, H. A., B. D. KESSING, G. M. WELLINGTON, and A. GRAYBEAL. 1996. Indo-Pacific echinoids in the tropical eastern Pacific. Coral Reefs **15**:133–142.

LESSIOS, H. A., and J. S. PEARSE. 1996. Hybridization and introgression between Indo-Pacific species of *Diadema.* Mar. Biol. **126**:715–723.

LI, W.-H. 1993. Unbiased estimation of the rates of synonymous and nonsynonymous substitution. J. Mol. Evol. **36**: 96–99.

———. 1997. Molecular evolution. Sinauer, Sunderland, Mass.

LI, W.-H., C.-I. WU, and C.-C. LUO. 1985. A new method for estimating synonymous and nonsynonymous rates of nucleotide substitutions considering the relative likelihood of nucleotide and codon changes. Mol. Biol. Evol. **2**:150–174.

LITTLEWOOD, D. T. J., and A. B. SMITH. 1995. A combined morphological and molecular phylogeny for sea urchins (Echinoidea: Echinodermata). Philos. Trans. R. Soc. Lond. B Biol. Sci. **347**:213–234.

LOHE, A. R., E. N. MORIYAMA, D. A. LIDHOLM, and D. L. HARTL. 1995. Horizontal transmission, vertical inactivation, and stochastic loss of mariner-like transposable elements. Mol. Biol. Evol. **12**:62–72.

LOUIS, C., and G. YANNOPOULOS. 1988. The transposable elements involved in hybrid dysgenesis in *Drosophila melanogaster.* Pp. 205–250 *in* N. MACLEAN, ed. Oxford surveys on eucaryotic genes. Oxford University Press, Oxford.

MCALLISTER, B. F., and J. H. WERREN. 1997. Phylogenetic analysis of a retrotransposon with implications for strong evolutionary constraints on reverse transcriptase. Mol. Biol. Evol. **14**:69–80.

MCDONALD, J. F. 1989. The potential evolutionary significance of retroviral-like transposable elements in peripheral populations. Pp. 190–205 *in* A. FONTDEVILA, ed. Evolutionary biology of transient unstable populations. Springer-Verlag, Berlin.

———. 1990. Macroevolution and retroviral elements. Bioscience **40**:183–191.

———. 1993. Transposable elements and evolution. Kluwer, the Netherlands.

———. 1995. Transposable elements: possible catalysts of organismic evolution. Trends Ecol. Evol. **10**:123–126.

MCHALE, M. T., I. N. ROBERTS, S. M. NOBLE, C. BEAUMONT, M. P. WHITEHEAD, D. SETH, and R. P. OLIVER. 1992. CfT-1: an LTR-retrotransposon in *Cladosporium fulvum,* a fungal pathogen of tomato. Mol. Gen. Genet. **233**:337–347.

MACKAY, T. 1986. Transposable element–induced fitness mutations in *Drosophila melanogaster.* Genet. Res. **48**:77–87.

MCMILLAN, W. O., R. A. RAFF, and S. R. PALUMBI. 1992. Population genetic consequences of developmental evolution in sea urchins (genus *Heliocidaris*). Evolution **46**: 1299–1312.

MARUYAMA, K., and D. L. HARTL. 1991. Evidence for interspecific transfer of the transposable element mariner between *Drosophila* and *Zaprionus.* J. Mol. Evol. **33**:514–524.

MICHAILLE, J. J., S. MATHAVAN, J. GAILLARD, and A. GAREL. 1990. The complete sequence of *mag,* a new retrotransposon in *Bombyx mori.* Nucleic Acids Res. **18**:674.

MIZROKHI, L. J., and A. M. MAZO. 1990. Evidence for horizontal transmission of the mobile element jockey between distant *Drosophila* species. Proc. Natl. Acad. Sci. USA **86**: 9216–9220.

MONTE, J. V., R. B. FLAVELL, and J. P. GUSTAFSON. 1995. WIS 2-1a: an ancient retrotransposon in the triticeae tribe. Theor. Appl. Genet. **91**:367–373.

MOORE, R. C. 1966. Treatise on invertebrate paleontology. Part U, Echinodermata 3. Geological Society of America, Lawrence, Kans.

MURATA, S., N. TAKASAKI, M. SAITOH, and N. OKADA. 1993. Determination of the phylogenetic relationships among Pacific salmonids by using short interspersed elements (SINEs) as temporal landmarks of evolution. Proc. Natl. Acad. Sci. USA **90**:6995–6999.

PALUMBI, S. R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. J. Exp. Mar. Biol. Ecol. **203**:75–92.

PAMILO, P., and O. BIANCHI. 1993. Evolution of the Zfx and Zfy genes: rates and interdependence between the genes. Mol. Biol. Evol. **10**:271–281.

ROBERTSON, H. M. 1997. Multiple mariner transposons in flatworms and hydras are related to those of insects. J. Hered. **88**:195–201.

ROBERTSON, H. M., and D. J. LAMPE. 1995. Recent horizontal transfer of a mariner transposable element among and between Diptera and Neuroptera. Mol. Biol. Evol. **12**:850–862.

ROSE, R. R., and W. F. DOOLITTLE. 1983. Molecular mechanisms of speciation. Science **220**:157–162.

RUBIN, G. M., M. G. KIDWELL, and P. M. BINGHAM. 1982. The molecular basis of P-M hybrid dysgenesis: the nature of induced mutations. Cell **29**:987–994.

SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.

SCHEINKER, V. S., E. R. LOZOVSKAYA, J. G. BISHOP, and M. B. EVGEN'EV. 1990. A long terminal repeat–containing retrotransposon is mobilized during hybrid dysgenesis in *Drosophila virilis.* Proc. Natl. Acad. Sci. USA **87**:9615–9619.

SIMMONS, G. M. 1992. Horizontal transfer of hobo transposable elements within the *Drosophila melanogaster* species complex: evidence from DNA sequencing. Mol. Biol. Evol. **9**:1050–1060.

SMITH, A. B. 1981. Implications of lantern morphology for the phylogeny of post-Paleozoic echinoids. Palaeontology **24**: 779–801.

———. 1984. Echinoid paleobiology. Allen and Unwin, London.

———. 1988. Phylogenetic relationship, divergence times, and rates of molecular evolution for Camarodont sea urchins. Mol. Biol. Evol. **5**:345–365.

———. 1989. RNA sequence data in phylogenetic reconstruction: testing the limits of its resolution. Cladistics **5**:321–344.

———. 1992. Echinoderm phylogeny: morphology and molecules approach accord. Trends Ecol. Evol. **7**:224–229.

SMITH, A. B., and D. T. J. LITTLEWOOD. 1994. Paleontological data and molecular phylogenetic analysis. Paleobiology **20**: 259–273.

SMITH, A. B., B. LAFAY, and R. CHRISTEN. 1992. Comparative variation of morphological and molecular evolution through geologic time: 28s ribosomal RNA versus morphology in echinoids. Philos. Trans. R. Soc. Lond. B Biol. Sci. **338**: 365–382.

SMITH, M. J., J. D. G. BOOM, and R. A. RAFF. 1990. Single copy DNA distance between two congeneric sea urchin species exhibiting radically different modes of development. Mol. Biol. Evol. **7**:315–326.

SMITH, P. A., and V. G. CORCES. 1991. *Drosophila* transposable elements: mechanisms of mutagenesis and interactions with the host genome. Adv. Genet. **29**:229–300.

SPRINGER, M. S., and R. J. BRITTEN. 1993. Phylogenetic relationships of reverse transcriptase and RNAase H sequences and aspects of genome structure in the *gypsy* group of retrotransposons. Mol. Biol. Evol. **10**:1370–1379.

SPRINGER, M. S., E. H. DAVIDSON, and R. J. BRITTEN. 1991. Retroviral-like element in a marine invertebrate. Proc. Natl. Acad. Sci. USA **88**:8401–8404.

SPRINGER, M. S., N. A. TUSNEEM, E. H. DAVIDSON, and R. J. BRITTEN. 1995. Phylogeny, rates of evolution, and patterns of codon usage among sea urchin retroviral-like elements, with implications for the recognition of horizontal transfer. Mol. Biol. Evol. **12**:219–230.

TAKAHASHI, K., Y. TERAI, M. NISHIDA, and N. OKADA. 1998. A novel family of short interspersed repetitive elements (SINEs) from cichlids: the patterns of insertion of SINEs at orthologous loci support the proposed monophyly of four major groups of ciclid fishes in Lake Tanganyika. Mol. Biol. Evol. **15**:391–407.

TANDA, S., J. L. MULLOR, and G. CORCES. 1994. The *Drosophila tom* retrotransposon encodes an envelope protein. Mol. Cell. Biol. **14**:5392–5401.

USDIN, K., P. CHEVRET, F. M. CATZEFLIS, R. VERONA, and A. V. FURANO. 1995. L1 (line-1) retrotransposable elements provide a ''fossil'' record of the phylogenetic history of murid rodents. Mol. Biol. Evol. **12**:73–82.

VANDERWIEL, P. L., D. F. VOYTAS, and J. F. WENDEL. 1993. *Copia*-like retrotransposable element evolution in diploid and polyploid cotton (*Gossypium* L.). J. Mol. Evol. **36**:429–447.

VERNEAU, O., F. CATZEFLIS, and A. V. FURANO. 1998. Determining and dating recent rodent speciation events by using L1 (LINE-1) retrotransposons. Proc. Natl. Acad. Sci. USA **95**:11284–11289.

WALLACE, M. R., L. B. ANDERSON, A. M. SAULINO, P. E. GREGORY, T. W. GLOVER, and F. S. COOKINS. 1991. A de novo Alu insertion results in neurofibromatosis type 1. Nature **353**:864–866.

WOODRUFF, R. C. 1993. Transposable DNA elements and life history traits. I. Transposition of *P* DNA elements in somatic cells reduces the life span of *Drosophila melanogaster*. Pp. 218–229 *in* J. F. McDonald, ed. Transposable elements in evolution. Kluwer, the Netherlands.

THOMAS EICKBUSH, reviewing editor

Accepted March 24, 1999