

Single-Copy DNA Distance between Two Congeneric Sea Urchin Species Exhibiting Radically Different Modes of Development¹

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We have investigated the differences between nuclear genomes of two purportedly congeneric species of sea urchin that differ radically in early development. *Heliocidaris tuberculata* develops by means of a typical pluteus larva, whereas *H. erythrogramma* develops directly from an egg that is 100-fold the volume of the *H. tuberculata* egg. Reassociation kinetic analysis shows that the kinetic components of the genomic DNA from the two species are essentially the same. No single repeat component explains the 30% difference between the *H. erythrogramma* and *H. tuberculata* genomes. Reciprocal hybridization of tracer-labeled single-copy DNA fractions between these species indicates that ~50% of the single-copy DNA is sufficiently similar to form hybrids at standard hybridization criterion. Thermal denaturation profiles of the hybridized single-copy DNA sequence yields median (T_{50H}) values of 13.8°–16.5°C. This result suggests a divergence time of 10–13 Mya, which is comparable to divergence times between congeneric sea urchin species in other genera that do not differ significantly in development. Radical differences in early developmental processes can evolve rapidly between closely related forms.

Introduction

There are about 1,000 species of sea urchins extant. The majority develop via a feeding, planktotrophic larva that has an extended period of pelagic life. This echinopluteus larva then settles and undergoes a brief metamorphosis, which releases the juvenile sea urchin (Okazaki 1975). This form of development is primitive among sea urchins (Jagersten 1972; Strathmann 1978; Raff 1987). Approximately 20% of sea urchins, belonging to several different orders, have abandoned this mode of development in favor of direct development, in which a nonfeeding larva is produced. Instead of spending weeks of development from egg to metamorphosis as in typical development, some direct developers proceed from egg to juvenile adult in as few as 3½ d. A continuum of modifications of the pluteus has evolved, ranging from obligately feeding pluteus to loss of all larval features. These modifications include facultative planktotrophy (Emlet 1986), nonfeeding plutei (Okazaki 1975; Olsen et al., accepted), armless nonfeeding larvae (Williams and Anderson 1975; Amemiya and Tsuchiya 1979; Parks et al. 1989), and brooded embryos that lack a larval stage (Barker 1983; Schatt 1985). The changes resulting in direct development are expressed as a pattern of heterochronies, in which many features of indirect larval development are deleted or abbreviated, and features of adult development are initiated early and are accelerated

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in rate. The evolution of direct development is accompanied by changes in oogenesis. Species with typical feeding plutei produce small eggs (diameter $\sim 100 \mu\text{m}$), whereas the nonfeeding forms have very large eggs (diameter 300–1,200 μm) (Emlet et al. 1987).

The present analysis examines the reassociation kinetics and single-copy DNA hybridization between two species of sea urchins in the same genus. *Heliocidaris tuberculata* develops via a typical pluteus larva, whereas *H. erythrogramma* produces a nonfeeding larva with no arms and exhibiting extensive direct development. Both species are common members of the shallow-water fauna on the east coast of Australia. They reproduce at the same time of year and are apparently similar in adult biology and habitat. The genome sizes, as determined by flow cytometry (Raff et al., accepted), of these species are 1.3 pg for *H. erythrogramma* and 0.95 pg for *H. tuberculata*.

The enormous differences in gametes and in development between the two *Heliocidaris* species are comparable to differences expected between animals at class or even phylum level and are not typical of animals belonging to the same genus. The question then arises of just how distant these two species really are. There are two possibilities. First, the two species are misclassified and are really very distant from each other. In such a case the great similarities of adult morphology would be posited as due either to extreme conservatism or to a high degree of convergence between two distant echinoid lineages. On the basis of examination of related euechinoids, this appears to be an unlikely explanation (Smith 1988). The second possibility is that these species are closely related and that radical changes in early development have occurred since species divergence. The question then is, How long has that been?

In the present study, we examined two aspects of the evolution of the genomes of the two *Heliocidaris* species. We asked whether the roughly 30% difference in genome sizes between the two (Raff et al., accepted) can be attributed to a recent obvious amplification or deletion of part of the genome, and we asked how related is the single-copy DNA in the two genomes. Reassociation kinetic analysis of genomic DNA from the two species shows no significant difference in classes of kinetic components. Thus, the genome size difference cannot be explained by, for example, a recent saltatory amplification of a specific repeat DNA family. Studies on the thermal stability of single-copy DNA hybrids show that these species have been separated ~ 10 –13 Myr, a divergence time comparable to that of other congeneric sea urchin species that do not differ significantly in development. This fairly recent divergence suggests that radical changes in gametogenesis and development can occur rapidly in the evolution of species.

Methods

Reassociation Kinetic Analysis of Genomic DNAs

DNA was prepared from sperm by a modification of the method of Blin and Stafford (1976). Sperm were suspended (1:100 v/v) in 0.2 M Tris-HCl (pH 8.0), 0.2 M Na_2EDTA . Proteinase K (Sigma) was added to 120 $\mu\text{g}/\text{ml}$. After complete dispersion of the proteinase K, the sperm were lysed by adding sodium dodecyl sulfate (SDS) to 1% (w/v) and were incubated overnight at 37°C with gentle swirling. Suspensions were extracted three times with an equal volume of buffer-equilibrated phenol:sevag (24:1 chloroform:isoamyl alcohol) and were dialyzed for 3 d against TE (0.02 M Tris-HCl pH 8, 0.02 M Na_2EDTA). After adjustment to 0.1 M NaCl, RNase A was added to 100 $\mu\text{g}/\text{ml}$, and the suspension was incubated for 2 h at 37°C. Proteinase K was added to 100 $\mu\text{g}/\text{ml}$, SDS was added to 1%, and the DNA preparation was incubated overnight at 50°C. The DNA suspension was extracted with buffer-equili-

brated phenol:sevag and was dialyzed for 3 d against 0.03 M Na acetate, 1 mM EDTA buffer at pH 7. The solution was adjusted to 0.3 M Na acetate, and the DNA was wound from the interface after an overlaying with 2 vol 95% ethanol. Genomic DNA was prepared from sperm of 12 individual *H. erythrogramma* and two individual *H. tuberculata*.

The DNA was sheared in a Virtis homogenizer at 50,000 rpm according to a method described elsewhere (Britten et al. 1974). An aliquot of the randomly sheared DNA from each species was end-labeled with ^{32}P by polynucleotide kinase (Pharmacia) by using a gamma-labeled ^{32}P -ATP (4,500 Ci/mM; Amersham) after treatment of the DNA with bacterial alkaline phosphatase (Sigma) according to a method described elsewhere (Smith et al. 1980). The labeled DNA was added to an excess of unlabeled DNA prior to denaturation and reassociation reactions.

The average length of the randomly sheared DNA was estimated by electrophoresis on denaturing alkaline agarose gels (McDonnell et al. 1977), as well as on non-denaturing gels. *Hinf*I-restricted pBR322 DNA was used as a size standard. Median length was determined from gels, stained with ethidium bromide in the case of unlabeled DNA and by autoradiography on Kodak XK-1 (BB) film in the case of end-labeled DNA fragments. For both species, the median size of the unlabeled DNA fragment was 200 nucleotides (nt), and the ^{32}P -labeled DNA was 150 nt.

DNA reassociation reactions were performed either in 0.12 M PB (PB is equimolar Na mono- and dibasic phosphate pH 6.8) at 60°C or in 0.41 M phosphate buffer at 64°C according to a method described elsewhere (Smith et al. 1982). A fivefold rate-acceleration factor was used for the high-phosphate reactions (Britten et al. 1974). DNA reassociation reactions were assayed by hydroxyapatite (HAP) chromatography (Britten et al. 1974). The data were analyzed for multiple second-order reaction components by a least-squares fitting procedure (Pearson et al. 1977).

DNA Hybrid Melting Curves

Sheared genomic DNA from both *Heliocidaris* species was end-labeled with ^{32}P -ATP after being treated with alkaline phosphatase as outlined above. The labeled DNA was denatured and reassociated to a Cot of 137,256 for *H. tuberculata* or 69,888 for *H. erythrogramma*. The reassociated DNA was passed over a HAP column at 60°C in 0.12 M PB, 0.1% SDS. Duplex DNA was harvested from the columns in 0.5 M PB, dialyzed against 0.3 M Na acetate buffer, precipitated with 2 vol ethanol, air-dried, and taken up in 0.12 M PB. These DNA samples were denatured and reassociated to Cot 1,782 for *H. tuberculata* and Cot 1,802 for *H. erythrogramma*. These Cot values are near the $\text{Cot}_{1/2}$ for the single-copy component appropriate to each species (see fig. 1 and table 1). The DNA was chromatographed on HAP at 60°C in 0.12 M PB, 0.1% SDS, and the unreassociated single-copy DNA was collected.

DNA hybridization reactions were performed essentially as were the reassociation reactions outlined above. The mass excess of heterologous driver DNA was greater than $10^4:1$ in all cases. The amount of tracer-labeled single-copy DNA in hybrid DNA molecules at each Cot point was determined by HAP chromatography.

Thermal denaturation profiles for single-copy DNA reassociated either with homospecific DNA or with heterospecific genomic DNA was determined by HAP chromatography. Single-copy DNA labeled with ^{32}P was denatured in the presence of both mass and sequence excess of total genomic DNA. This mixture was reassociated, hybridized, to a genomic DNA driver Cot value $>10,000$. The reaction mixture was adjusted to 0.12 M PB, 0.01% SDS and was loaded on a HAP column in that buffer at 55°C. The column temperature was increased in ~ 3 -degree-C increments. At each

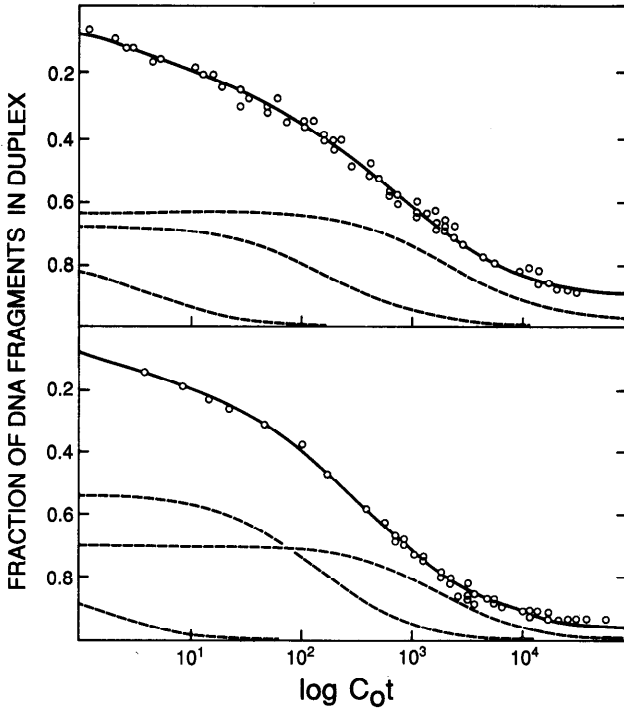


FIG. 1.—Reassociation kinetics of *Heliocidaris* species DNA. Upper panel, *H. erythrogramma*. Lower panel, *H. tuberculata*. The solid lines indicate the best-fit line for three frequency components in each genome; the dashed lines indicate each of those components. See table 1 for the statistical analyses of the reaction components.

temperature increment, the column was washed with buffer to elute denatured DNA. A plot of the accumulated fraction of the tracer-labeled DNA eluted from the HAP column provides the thermal denaturation profile for the reassociated or hybridized DNA. The temperature at which 50% of the hybridized or reassociated tracer is eluted is the melting or denaturation temperature (T_m). The median divergence (T_{50H}) is an estimate of the T_m of all potentially hybridizable sequences (Kohne 1970; Hall et al. 1980; Smith et al. 1982). Smith et al. (1982) have shown in sea star single-copy DNA hybridization analyses that, if the hybridization temperature is lowered to 50°C, there is not only an increased amount of hybridization but, more important, estimated T_{50H} values are the same as those estimated when duplex is formed at 60°C. Here we present both T_m and T_{50H} values.

Results

Reassociation Kinetics of *Heliocidaris erythrogramma* and *H. tuberculata* Genomes

The reassociation reactions of *H. erythrogramma* and *H. tuberculata* genomic DNAs are shown in figure 1. Both species are similar in the overall frequency composition of their genomic DNA. Both have moderately sized ($\sim 20\%$ of the haploid genome) middle repetitive components with average reiteration frequencies in the hundreds. Both species have large, slow, repetitive-sequence components with frequencies averaging ≤ 10 copies/haploid genome. This component has a large number of families of varying reiteration frequency. The range of repeat-family frequencies in

Table 1
Reassociation Kinetics of *Heliocidaris* Species DNA

SPECIES	MIDDLE REPEAT		SLOW REPEAT		SINGLE COPY		FINAL %	RMS ^a %	N ^b
	%	Cot _{1/2} ^c	%	Cot _{1/2} ^c	%	Cot _{1/2} ^c			
<i>H. erythrogramma</i> . . .	22.4	5.2	39.4	298.5	27.5	4329 ^d	6.3	1.9	63
	20.5	4.8	30.8	208.4	35.3	2331 ^e	8.1	2.0	60
<i>H. tuberculata</i>	21.1	3.0	27.8	156.3	41.6	776 ^d	5.7	0.9	58
	17.7	1.7	45.6	137.2	30.0	1799 ^f	3.5	1.4	56

^a Root mean square error of the data points to the least-squares best-fit solution.

^b Number of data points used in the least-squares solution.

^c Reciprocal of the second-order reaction rate, i.e., the Cot value at which one-half of the frequency component has reassociated.

^d None of the parameters in the least-squares analyses were fixed.

^e Single-copy DNA second-order reaction rate was fixed at a value appropriate for the haploid genome size of 1.3 pg. The expected single-copy reassociation rate was estimated for a DNA driver length of 200 nt and a tracer length of 150 nt (Chamberlin et al. 1978). This fit is illustrated in the upper panel of fig. 1.

^f Both the single-copy reaction rate and component size were fixed in the least-squares analysis. The rate was estimated for a genome size of 0.95 pg with a DNA driver fragment length of 200 nt and a DNA tracer length of 150 nt. This solution is illustrated in the lower panel of fig. 1.

this component makes an identification of the size and reassociation rate of the single-copy component statistically difficult.

With independent estimates of the haploid genome size (Raff et al., accepted) the rate of reassociation of single-copy DNA can be predicted (reviewed in Davidson 1986). Table 1 provides two solutions to the reassociation reactions for each species. All of the solutions are acceptable fits to the data. The root mean square deviations of points from the analytically fit lines are <2.0% for *H. erythrogramma* and <1.4% for *H. tuberculata*. In unconstrained analyses, the observed single-copy reassociation rate for *H. erythrogramma* is approximately two times the rate expected at that genome size, whereas in *H. tuberculata* the observed single-copy reassociation rate is approximately one-half the expected rate. If the second-order reaction rate of the single-copy component of *H. erythrogramma* is fixed at the value appropriate to the genome size, the size of single-copy component is estimated to be 35% (fig. 1 and table 1). The size of the single-copy component of *H. tuberculata* was fixed at 30% when the rate was set at a value consistent with the haploid genome size (fig. 1 and table 1).

Genomic Distance between *H. erythrogramma* and *H. tuberculata*

We have hybridized, reciprocally, single-copy tracer DNAs prepared from both *H. erythrogramma* and *H. tuberculata*. Both the reassociation of the single-copy fractions with their parent species and the hybridization reactions of these tracers with the heterologous DNA are shown in figures 2 and 3. An aliquot of each of the isolated putative single-copy DNA fractions was reassociated with a mass excess (>10⁴:1) of sheared total genomic DNA from its parent species to verify that they contained only single-copy DNA sequences. The reassociation reactions and the data (figs. 2 and 3 and table 2) were analyzed for single or multiple second-order reaction components. These data are best fit by using a single second-order reaction component. The reassociation rates for the tracers reacting with their parent DNAs, after appropriate length corrections, are within a factor of two of the expected single-copy rate (tables 1 and 2). In each case the extent of reaction of the prepared tracer is decreased, to 67% for

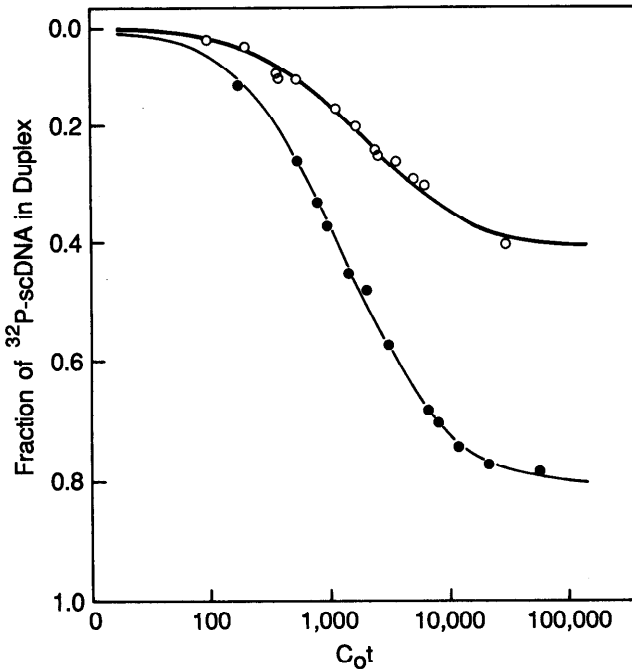


FIG. 2.—Hybridization kinetics of *Heliocidaris tuberculata* single-copy DNA. Labeled single-copy DNA from *H. tuberculata* was reassociated with a mass excess of randomly sheared genomic DNA from *H. tuberculata* (black dots) or hybridized with a mass excess of randomly sheared genomic DNA from *H. erythrogramma* (circles). The solid lines are the computer-analyzed best-fit lines for a single second-order reaction component.

H. erythrogramma and to 77.8% for *H. tuberculata*. Lowered reactivity of isolated single-copy fractions is a common phenomenon (Hall et al. 1980; Smith et al. 1982).

In the hybridization reactions, where single-copy DNAs are reacted with the genomic DNA from the heterologous species, the corrected extent of hybridization of the single-copy DNA is 47% of the *H. erythrogramma* single-copy tracer (0.315/0.678) and is 48.5% of the *H. tuberculata* single-copy tracer (0.377/0.778). Again, the rate of hybridization with heterologous tracers is less than a factor of two different from that seen for homologous tracer (table 2), verifying the unique sequence character of hybridized fragments.

Estimations of phylogenetic relatedness that are based on DNA hybridization analyses depend on both the extent of cross-reaction of single-copy tracer DNAs and the T_m 's of hybrid duplexes. Reciprocal hybridizations provide an internal control for the accuracy of the divergence estimate. In figures 4 and 5 we have plotted the HAP thermal denaturation profiles for single-copy tracer DNAs reacted with both the parent species and the heterologous species. Two distance measures can be extracted from these curves: (1) the difference in the T_m 's of the single-copy tracer reacted with the parent and heterologous species ($-dT_m$) and (2) the T_{50H} , which is the temperature at which 50% of the reactable tracer would be hybridized. We have estimated the T_{50H} value of the accumulated fraction of the hybridized single-copy DNA as a function of elution temperature (figs. 4 and 5 and table 2).

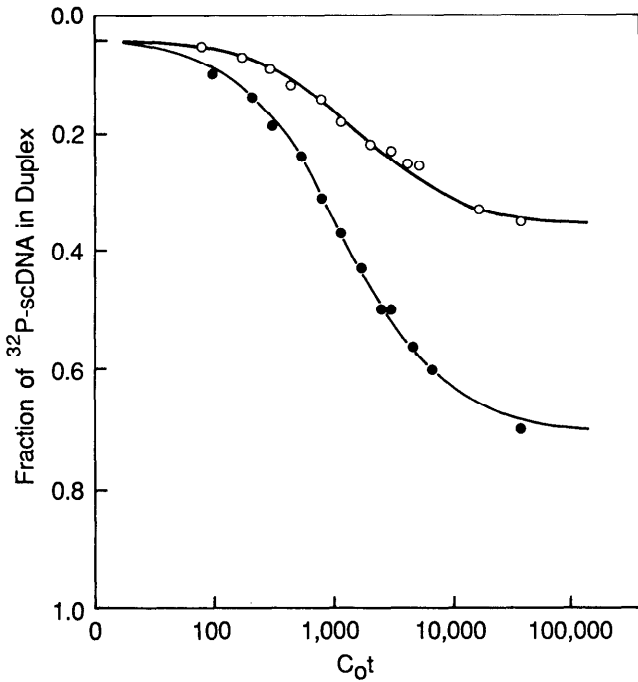


FIG. 3.—Hybridization kinetics of *Heliocidaris erythrogramma* single-copy DNA. Labeled single-copy DNA from *H. erythrogramma* was reassociated with a mass excess of randomly sheared genomic DNA from *H. erythrogramma* (black dots) or hybridized with a mass excess of randomly sheared genomic DNA from *H. tuberculata* (circles). The solid lines are the computer-analyzed best-fit lines for a single second-order reaction component.

Discussion

Genome Size as a Correlate of Phylogenetic Relationship and Developmental Mode

Although the genome sizes of different species within certain genera can vary as much as 10-fold (Rees and Hazarika 1969; Jones and Brown 1976), examples of such

Table 2
Hybridization of *Heliocidaris* Species Single-Copy DNA

Driver DNA/Tracer DNA ^a	Fraction Hybridized	Rate (M/s)	RMS (%)	T _m ^b (degrees C)	-dT _m ^c (degrees C)	T _{50H} ^d (degrees C)
<i>H. erythrogramma</i> / <i>H. erythrogramma</i>	0.670	.000791	1.35	74.5		
<i>H. erythrogramma</i> / <i>H. tuberculata</i>	0.377	.000461	1.23	68.5	6.0	13.8
<i>H. tuberculata</i> / <i>H. tuberculata</i>	0.778	.000745	1.08	76.0		
<i>H. tuberculata</i> / <i>H. erythrogramma</i>	0.315	.000572	0.95	68.5	7.5	16.5

^a Genomic driver DNA and tracer-labeled single-copy DNAs were prepared from two *H. tuberculata* males and from 12 *H. erythrogramma* males.

^b Temperature at which half of the hybridized DNA is thermally denatured.

^c Difference in the observed T_m values between homologous and heterologous tracer single-copy DNA hybridized with the noted driver DNA.

^d Median divergence, estimated by extrapolation of the linear portion of the heterospecific thermal elution profile to a value of 50% tracer hybridized.

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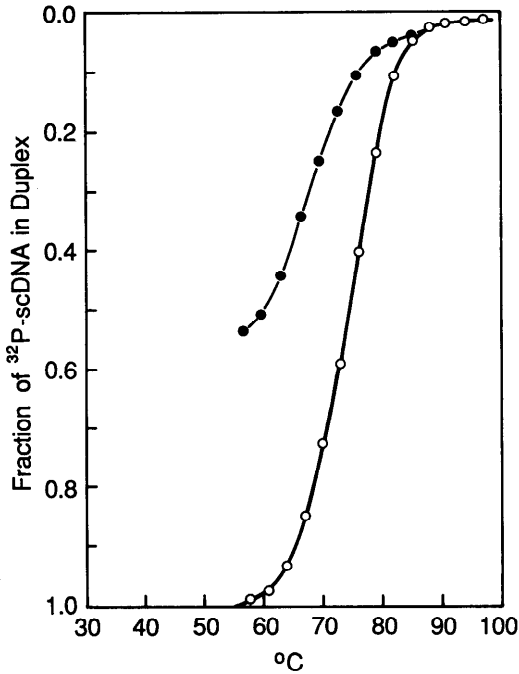


FIG. 4.—Thermal denaturation profile of *Heliocidaris tuberculata* single-copy DNA hybridized to a mass excess of *H. erythrogramma* genomic DNA. The thermal denaturation profile of *H. tuberculata* scDNA hybridized to *H. erythrogramma* genomic DNA (black dots) is compared with the thermal denaturation profile of *H. erythrogramma* scDNA reassociated with an excess of genomic DNA from that species (circles).

variation have not been recorded in echinoids. Echinoid genome sizes vary among echinoid orders over approximately a twofold range (Raff et al., accepted).

Although the sample size of direct developers is small, among the regular euechinoids there does appear to be a correlation between developmental mode, sperm head length and proportions, and genome size. The representatives of the euechinoids for which there are data have genome sizes ranging from 0.83 to 0.95 pg, except for two direct developers, *Holopneustes inflatus* (1.4 pg) and *Heliocidaris erythrogramma* (1.3 pg). *Heliocidaris tuberculata*, which develops typically through a pluteus larvae, has a haploid genome size of 0.95 pg (Raff et al., accepted). Like *Heliocidaris erythrogramma*, *Holopneustes*, a direct developer with a very large egg, has, for this order, longer than typical sperm head and a larger than average genome size.

The DNA reassociation curves do not present a simple explanation for the difference in genome size between *Heliocidaris tuberculata* and *Heliocidaris erythrogramma*. Clearly, *Heliocidaris erythrogramma* does not have any single, large, simple repeat component which makes up 30%–40% of the mass of the DNA. This scenario would be expected if the difference between these genomes had resulted from a recent large saltatory event (Britten and Kohne 1968). Whatever the genomic events that resulted in the differences in the nuclear DNA content between these species, it is clear that the kinetic components within the genomes are now similar in terms of resolvable reiteration frequencies and component sizes. However, these kinetic analyses do not address the nature of the sequences represented within the repeat families, and, since little is known about the rates of addition or deletion of specific frequency components and genomic compartments (for discussion, see Dover 1987), no firm con-

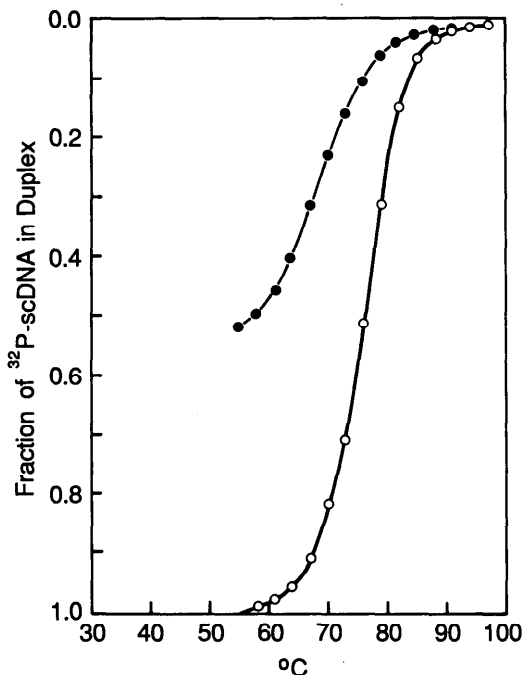


FIG. 5.—Thermal denaturation profile of *Heliocidaris erythrogramma* single-copy DNA hybridized to a mass excess of *H. tuberculata* genomic DNA. The thermal denaturation profile of *H. erythrogramma* scDNA hybridized to *H. tuberculata* genomic DNA (black dots) is compared with the thermal denaturation profile of *H. tuberculata* scDNA reassociated with an excess of genomic DNA from that species (circles).

clusions concerning divergence between these species can be made on the basis of reassociation studies alone.

Genetic Distance between *Heliocidaris tuberculata* and *Heliocidaris erythrogramma*

Sperm from only two *Heliocidaris tuberculata* males was available for these analyses. Consequently, the DNA extracted from these two individuals probably does not reflect the full range of polymorphic single-copy sequences in the population. This supposition is strengthened by the observation that the T_m of single-copy DNA prepared from two *Heliocidaris tuberculata* individuals is 1.5 degrees C higher than that for single-copy DNA prepared from 12 *Heliocidaris erythrogramma* males. This difference in T_m causes an elevation in the calculated T_{50H} values, since both reciprocal hybridizations have equivalent T_m 's of 68.5°C. The difference due to intrapopulational polymorphisms is significant but is small relative to the difference between species. Estimates of divergence time from thermal denaturation data depend on two factors. The first is an estimate of the percentage of nucleotide mismatch represented by a 1-degree-C difference in T_m of DNA duplexes. The second is an independent estimate of the rate of nucleotide change per unit time, an estimate based on measurements of thermal denaturation between species of known divergence time (actually an estimate from the fossil record). The extensive sequence hybridization studies of Britten and co-workers has resulted in the estimation of a divergence rate of 0.66% nt change/Myr for sea urchins (reviewed by Britten 1986). This estimate is based on an equivalence value of 1% nt mismatch to a 1-degree-C depression in T_m . Recent studies indicate

that a 1-degree-C depression in T_m represents a 1.7% mismatch (Caccone et al. 1988). However, we use the Britten measure because the estimates of sea urchin species divergence times are uncertain to the same degree (Smith 1988), and because, by using the Britten estimates, we can directly compare *Heliocidaris* DNA differences and estimated divergence times with those published for species of *Strongylocentrotus*.

If we use a median divergence of 13.8, our estimate for the length of time since a last common ancestor for the two *Heliocidaris* species is 10.5 Myr [(13.8/0.66)/2]. If we use a median divergence value of 16.5, 12.5 Myr have elapsed since the two species diverged. Both of these values are less than the estimated divergence between *S. franciscanus* and *S. purpuratus* but are greater than the divergence estimate for *S. purpuratus* and *S. droebachiensis* (Hall et al. 1980).

It had not been clear a priori how long it took to evolve the large number of differences in early development between *Heliocidaris erythrogramma* and *Heliocidaris tuberculata*. The simple heterochrony model does not describe all of the changes that have occurred in the evolution of direct development in *Heliocidaris erythrogramma*, and the larva is not merely a degenerate pluteus (Raff 1988). Early development has been reorganized in very basic ways. These include cell cleavage rate (Parks et al. 1988), cleavage pattern (Williams and Anderson 1975; Wray and Raff 1989), mode of cell determination, patterns of cell lineage differentiation, cell fates (Wray and Raff 1989), and formation of a wrinkled blastula (Williams and Anderson 1975).

Egg size also evolves. All known direct-developing echinoids produce significantly larger eggs than do indirect-developing species (Emlet et al. 1987; Raff 1987). Evolution of this feature may be a prerequisite to the evolution of nonfeeding direct-developing larvae, because sufficient food reserves and mass will be required for rapid direct development of a juvenile sea urchin. However, there are other consequences as well, including a greatly reduced nucleo-cytoplasmic ratio. This change has had a profound affect on cell cleavage dynamics (more rounds of cell cleavage before reaching a nucleo-cytoplasmic ratio such that cleavage ends), and it results in a much larger cell number at gastrulation in *Heliocidaris erythrogramma* than in *Heliocidaris tuberculata* (Parks et al. 1988). There appear to be other changes in the egg that affect fertilization, because the sperm heads of *Heliocidaris erythrogramma* are much larger than those of *Heliocidaris tuberculata* (Raff et al., accepted). Finally, the major storage proteins of the egg are very different in the two species, with the protein of *Heliocidaris erythrogramma* being different than the major yolk protein found in all previously studied sea urchins (Scott et al. 1990).

The single-copy DNA divergence data indicate that the two living *Heliocidaris* species represent recently diverged lineages. Although we cannot assess either the order of changes or the time required to achieve specific characters associated with direct development in the *erythrogramma* lineage, the relatively recent separation of the two lineages implies that considerable genetic differentiation can occur rapidly.

Although the early developments of *Heliocidaris erythrogramma* and *Heliocidaris tuberculata* differ in numerous ways, the adults are similar in morphology and have been seen by taxonomists as being members of the same genus. Only two living species are recognized. The genus *Heliocidaris* has a fossil record dating back ~30 Myr (Philip 1965) and is endemic to the Australian region but appears to be closely related to other members of the family Echinometridae. In fact, the Japanese species *Anthocidaris crassispina*, which develops indirectly via a typical pluteus, was formerly placed in the genus *Heliocidaris*. The close relationship both between the two *Heliocidaris* species and of both of them to *Strongylocentrotus* is borne out by 18S rRNA sequence data (Raff et al. 1988). All species of *Strongylocentrotus* develop indirectly.

The present study demonstrates that radical changes in development, oogenesis, and spermatogenesis can occur in relatively brief spans of evolutionary time. A thorough comparative analysis of the coding sequences in nuclear DNA of these species would probably display little difference. The kinds of differences that result between these species more likely arise from patterns of control and timing of expression of specific gene sets.

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