

attempted to demonstrate repair replication using the method of REGAN et al.¹¹ Figure 2 shows the result of a preliminary experiment using UV-light. *Pol* + bacteria were grown in medium containing ³H-thymidine for several generations to label the DNA uniformly. The cells were removed from radioactive medium and resuspended in medium containing BUdR or thymidine, where they were incubated for 5 min at 37°C, then chilled, centrifuged, resuspended in water, and irradiated with UV with bulk of radiation at 254 nm. They were then returned in darkness to the appropriate medium, where they were allowed to repair for 1 hour to incorporate BUdR into stretches of previously labeled DNA. Isopycnic centrifugation of this DNA at this stage confirmed that the BUdR molecules were actually incorporated into the cell during the recovery phase. Earlier experiments had also suggested that a semiconservatively synthesized BUdR-containing daughter strand (in the unirradiated control) hydrogen bonded to the labeled strand rendered the parent strand more susceptible to breakage by 313-nm irradiation. Therefore, all cells were grown for an additional generation (1.0 h in this medium) before photolysis. The cells were converted to protoplasts, then exposed to 313-nm irradiation (1.0×10^5 erg mm⁻²). The shift in single strand molecular weight was most pronounced in the DNA from cells repaired in the presence of BUdR, although some breakage also occurred both in cells repairing in thymidine and in unirradiated cells incubated in BUdR. The weight average molecular weights from this and the following experiment, summarized in the Table, are calculated from summation of the equation $d = aM^x$ for all fractions, where d = fractional distance sedimented and M = molecular weight¹⁵. The values of a and x (0.0145 and

0.316, respectively) were derived from sedimentation data from DNA samples of known molecular weight. When a similar experiment was carried out using X-irradiation (Figure 3), no such decrease in the average molecular weight was observed. Since this dose of X-ray in our system results in breakage of DNA down to at most half of the unirradiated weight average molecular weight, if the fraction of repaired regions photolysed were 0.33 to 0.5, as calculated by REGAN et al.¹¹ in the case of UV, this should have yielded a readily apparent change in the sedimentation pattern. We have also used the radiation-resistant mutant B/r and a larger but acceptable X-ray dose (30 kr) and again failed to detect appreciable photolysis in cells repaired in BUdR. The dose of 313-nm irradiation was varied in several experiments between 0.23×10^5 and 2.3×10^5 erg mm⁻² without enhanced resolution.

A reasonable interpretation of these data is that DNA repair synthesis is a necessary step in the closure of strand breaks caused by X-ray in *E. coli*, but that it involves much shorter segments of DNA than in UV excisional repair. This explanation is also consistent with the failure by others, except at the highest doses, to detect repair synthesis by isopycnic centrifugation in CsCl gradients¹⁶.

Zusammenfassung. Nachweis, dass bei einer *E. coli*-Mutante mit fehlender DNS-Polymerase eine DNS-«Repair»-Synthese möglicherweise ein notwendiger Schritt vor dem Schliessen der von Röntgenstrahlen verursachten Chromosomenbrüche ist.

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Change in average molecular weight caused by 313-nm irradiation (1.0×10^5 erg mm⁻²)

Condition	Pre. 313 nm M.W. ($\times 10^{-6}$)	Post 313 nm M.W.	Reduction of M.W. (%)
UV, thymidine	85.1	72.7	14.5
no UV, BUdR	73.2	62.6	14.4
UV, BUdR	93.6	49.2	47.4
X-ray, thymidine	97.2	83.2	14.4
no X-ray, BUdR	99.3	80.1	19.3
X-ray BUdR	104.9	84.0	19.9

Nuclear DNA Amounts in Polychaete Annelids

Recent work on evolutionary trends in the specific amount of nuclear DNA has been limited mainly to vertebrate groups¹⁻³. Much less is known about invertebrate genome sizes⁴. We report here the results of measurements made on 36 polychaete species. These worms constitute an invertebrate group of particular evolutionary interest. Morphologically some polychaetes are very close to the postulated ancestral body plan of the segmented coelomates. This basic pattern has been modified in various ways by adaptation to diverse marine habitats. There are free-swimming, crawling, burrowing, and temporarily or permanently sessile species, ranging from generalized to very specialized morphology.

Nuclear DNA amounts were determined by either of 2 methods. Data on some species are based on microdensitometric determinations of Feulgen dye content of individual somatic nuclei, others have been obtained by fluorometric quantitation of the DNA of a known number

¹ R. HINEGARDNER, *Am. Nat.* 102, 517 (1968); *Am. Nat.*, in press (1972).

² S. OHNO, *Evolution by Gene Duplication* (Springer, Berlin 1970).

³ K. BACHMANN, O. B. GOIN and C. J. GOIN, *Brookhaven Symp.* 23, in press (1972).

⁴ A. H. SPARROW, H. J. PRICE, and A. G. UNDERBRINCK, *Brookhaven Symp.* 23, in press (1972).

¹⁵ P. DOTY, B. MCGILL and S. RICE, *Proc. natn. Acad. Sci., USA* 44, 432 (1958).

¹⁶ This investigation was supported by USPHS research grant No. 5T01-GM00516-11, VA Hospital research grant and AEC contract No. AT (40-1) 3631.

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Haploid nuclear DNA content of polychaetes. Fluorometric determination on sperm cells and microdensitometric determination on Feulgen stained somatic cells

	Haploid DNA content in picograms		SE (%)	No. of slides
	Fluorometric	Microdensitometric		
Order Amphinomorpha				
Family Amphinomidae				
<i>Pseuderythoe ambigua</i>		2.4	5.5	3
Order Eunicemorpha				
Family Onuphidae				
<i>Onuphis eremita oculata</i>		1.7	6.7	8
<i>Onuphis magna</i>		1.2		1
<i>Onuphis sp.</i>		1.7	7.3	2
<i>Diopatra cuprea</i>		2.0	3.0	16
Family Lumbrineridae				
<i>Lumbrineris tenuis</i>		2.4	6.6	6
<i>Ninoe nigripes</i>	5.3			
Order Phyllodoceomorpha				
Family Phyllodocidae				
<i>Nereiphylla peretti</i>		2.7	1.6	2
Family Polynoidae				
<i>Lepidonotus squamatus</i>	1.5			
<i>Lepidonotus sublevis</i>	2.2			
Family Hesionidae				
<i>Podarke obscura</i>		1.6	9.9	3
Family Nereidae				
<i>Nereis succinea</i>	2.1	2.2	4.0	13
<i>Laconereis culveri</i>		0.8	10.1	5
Family Nephtyidae				
<i>Nephtys incisa</i>	7.2			
<i>Nephtys sp.</i>	2.2			
Family Glyceridae				
<i>Glycera americana</i>		3.5	1.9	3
Order Spiomorpha				
Family Orbiniidae				
<i>Scoloplos fragilis</i>		2.3	4.7	6
<i>Scoloplos rubra</i>		3.1	3.4	3
Family Cirratulidae				
<i>Cirriformia filigera</i>		1.0	2.2	19
<i>Cirriformia luxuriosa</i>	3.4			
<i>Cirratulus grandis</i>	0.7			
<i>cirratulid</i>		1.0		1
Family Chaetopteridae				
<i>Chaetopterus variopedatus</i>	1.0			
Family Paraonidae				
<i>Aricidea fragilis</i>		4.6	2.3	3
Order Drilomorpha				
Family Arenicolidae				
<i>Arenicola cristata</i>		0.9	3.3	13
Family Maldanidae				
<i>Clymenella mucosa</i>		2.7	2.2	15
Family Scalibregmidae				
<i>Scalibregma inflatum</i>	4.0			
Order Terellomorpha				
Family Pectinariidae				
<i>Pectinaria gouldii</i>	1.4	1.3	7.2	6
Order Serpulimorpha				
Family Sabellidae				
<i>Branchiommia nigromaculata</i>		1.3	1.2	3
<i>Myxicola infundibulum</i>	3.1			
Unidentified different species	1.1			
	1.9			
	2.3			
	2.8			
	2.8			
	2.9			

of sperm cells. Details of both methods have been published elsewhere^{5,6}. Feulgen determinations on polychaete nuclei were standardized by including *Cirratuliformia filigera*, *Diopatra cuprea*, and *Arenicola cristata* in every series of measurements. Two species were assayed by both methods and allow a calibration of the Feulgen data in absolute terms. This calibration was refined further using data on crustaceans and Feulgen values of vertebrate species with known amounts of DNA. All calibrations agree closely and the *pico*-gram scale is probably accurate to within 10% or better.

The genome sizes expressed as DNA per haploid nucleus are listed in the Table in taxonomic sequence⁷. The 10-fold range from 0.7 μ g for *Cirratulus grandis* to 7.2 μ g for *Nephtys incisa* is comparable to the ranges found for the more variable vertebrate groups, i.e. teleosts and anurans. The frequency distribution of DNA contents is illustrated in the figure. Here again, as in some vertebrate groups³ a logarithmic normal curve describes the observed distribution rather well. This may be taken to indicate an evolutionary history based on many cumulative smaller events. Occasional 1:2 relationships within a single family of polychaetes (Lumbrinereidae, Sabellidae) may reflect recent polyploid speciation. Speciation by polyploidization need not affect the smoothness of the distribution, if the doubled state is transitory and soon reduced by deletions.

Like many vertebrate groups the polychaetes show a smooth wide distribution of nuclear DNA amounts corresponding to a continuous wide variation of morphological types. Species in individual families usually have similar DNA amounts correlating with closer morphological similarity. This relationship between genome size and morphological diversities is not based on a one-to-one correlation of DNA amount with any morphological parameter. The limited size of the sample precludes the

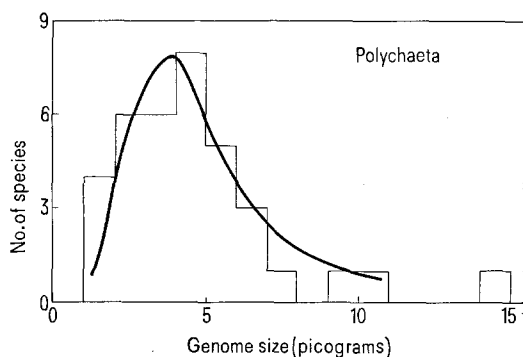
more detailed correlations which are already possible in vertebrates, where a striking correlation between small genome size and specialized morphology has been noted repeatedly^{1,3}. Among the polychaetes, the more generalized errant species (following DAY's separation⁸) have an average genome size of 2.5 μ g, while the more specialized sedentary species⁸ average 1.8 μ g DNA haploid. Among the latter, the very specialized filter and tentacular feeders alone average 1.6 μ g DNA per haploid nucleus. These figures suggest a correlation between small genomes and specialization but they are at the lower limit of statistical significance and only a larger sample could verify this correlation for polychaetes.

The range in DNA content found in the polychaetes encompasses that of the mammals even though the mammalian modal value of 4 μ g is twice as high as the polychaete mode of 2 μ g (Figure). It is understandable that reduction of genome size can lead to considerably smaller DNA amounts in annelids when compared to the more complex mammals. It is more difficult to understand why even a generalized polychaete with a large amount of evolutionary potential should have as much DNA as we find in some species. Apparently one of the features of the genome of more complex animal groups is a more stringent control on genome variability⁶. Certainly the amount of DNA is under selective control and the similarities in the patterns encountered in the polychaetes and the vertebrates are striking.

Zusammenfassung. Bestimmung des DNS-Gehalts pro Zellkern in 36 Polychätenarten ergeben eine Häufigkeitsverteilung der Genomgrößen, die durch eine logarithmische Normalverteilung beschrieben werden kann. Eine Korrelation zwischen adaptiver Spezialisierung und reduzierten Genomen, wie sie bei Wirbeltieren gefunden wird, ist auch bei Polychäten angedeutet.

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Diploid nuclear DNA amount of 36 species of polychaetes. The curve is a best fit logarithmic normal distribution for these data.

⁵ R. HINEGARDNER, *Analyt. Biochem.* 39, 197 (1971).

⁶ K. BACHMANN, *Chromosoma* 37, 85 (1972).

⁷ R. B. CLARK, *Chemical Zoology* (Eds. FLOKIN and SCHEER; Academic Press, New York 1969), vol. 4, p. 1.

⁸ J. H. DAY, *A Monograph of the Polychaeta of Southern Africa* (British Museum, London 1967).

⁹ The authors express their appreciation for help received from Dr. J. SIMON and D. DAUER in identifying polychaete species and discussing their results.

Endophenotype of Mysore (South India) Population of the Black Rat (*Rattus rattus* L.)

Karyological studies of *Rattus rattus* are interesting, contradictory and intriguing¹⁻³. BADR and BADR¹ reported 3 coexistent chromosome numbers namely 38, 42 and ± 54 . Occurrence of 38 chromosomes was reported by BIANCHI et al.⁴ in 2 South American populations. CAPANNA et al.⁵⁻⁷ claimed similar number in Italian, West European and African populations. Reports on the Asian populations¹ always showed 42 chromosomes. Karyolo-

gical studies of 3 separate populations of *Rattus rattus* of Mysore area (South India) showed that the diploid number is 38 (11 pairs of biarmed chromosomes, 7 pairs of acrocentrics + X and Y both being acrocentric). Further 25-30% of the first pair of chromosomes are heteromorphic. Comparison of the karyotype of Mysore population with those of Italian (CAPANNA et al., *MCN*. 10, 4 (1969), personal commun.) and South American and