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DNA Reassociation Kinetics and Chromosome Structure in the Crabs *Cancer borealis* and *Libinia emarginata*

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Abstract. DNA reassociation kinetics have been partly elucidated for the higher crabs *C. borealis* and *L. emarginata*, using calf thymus DNA as a standard. These crabs contain no detectable repeated DNA in the approximate multiplicity frequency range 2–100 copies, which is unusual for invertebrate DNAs. Each species contains a component renaturing at an intermediate rate, and also a very rapidly renaturing fraction. The very rapidly renaturing fraction is considerably larger than the cesium chloride-resolvable satellites of each species. The fraction reassociating at an intermediate rate includes sequences with a reiteration frequency of up to 9.0×10^4 copies. This is unusually high for invertebrate DNAs. The nearly exact correlation between kinetic complexity and independently determined haploid genome size leads to the conclusion that the most slowly renaturing sequences of both crab species are present only once per haploid genome. Therefore the chromatids of these species are uninemic structures, and there has been no detectable occurrence of polyploid speciation in the recent evolutionary history of either species.

Introduction

Physical separation of the two strands of DNA, followed by careful analysis of their reassociation under controlled conditions, has been shown to be a powerful tool for the analysis of chromosome structure (Laird, 1971), gene regulation (Britten and Davidson, 1969, 1971) and DNA evolution (for reviews, see Kohne, 1970; Laird, 1973; Davidson and Britten, 1973; Britten *et al.*, 1974). These reassociation reactions are generally observed either by analysis of hypochromicity in a spectrophotometer cuvette (Wetmur and Davidson, 1968) or by separation of reassociated double-stranded DNA from single-stranded DNA on calcium phosphate (hydroxylapatite) columns (Britten and Kohne, 1967).

Early in a study of dAT satellite DNA evolution in crustaceans, analyses of DNA thermal denaturation profiles from a number of crustacean species led to the prediction (Vaughn, 1972; Vaughn and Bachmann, 1973; Vaughn, 1974) that many higher crustaceans would contain a great deal more very rapidly reassociating DNA than is present in the classical satellite fractions, which are resolvable by cesium chloride centrifugation (Skinner, 1967; Skinner *et al.*, 1970). We thought that this might be the case for the following reasons. When DNA from

higher crabs of various families is denatured in a spectrophotometer cuvette, we often find that although *various* percentages of the respective genomes are represented by a dAT satellite, a *fixed* percentage is (A + T)-rich *regardless* of the percentage of dAT. These observations also hold when absolute DNA amounts are measured. One interpretation of these data would be that an ancestral organism contained that fixed percentage of (A + T)-rich DNA, presumably as dAT satellite, but that either a fraction of this has accumulated base substitutions and thereby become somewhat less (A + T)-rich, and/or has become dispersed throughout the genome in sequences so short that they denature at somewhat higher temperatures than pure dAT owing to the influence of adjacent sequences. In either case, we would anticipate that the percent of very rapidly reassociating DNA sequences would approximate the percent of the genome which is (A + T)-rich by thermal denaturation analysis, provided of course that divergence had not proceeded to the point where DNA homologies could not be detected by reassociation techniques.

If these hypothetical very fast fractions could indeed be verified, then it would be desirable to isolate them so that they could be directly analyzed. Both of these considerations led to the analyses of DNA renaturation kinetics reported in this communication. In addition, recent evidence suggesting that speciation by polyploidy may have occurred in some of the higher *Crustacea* (Bachmann and Rheinsmith, 1973) prompted us to inquire as to the feasibility of using DNA kinetic analysis as a tool to probe this interesting hypothesis.

Materials and Methods

Living male specimens of the crabs *Cancer borealis* (Infraorder *Brachyura*, Section *Cancridea*) and *Libinia emarginata* (Infraorder *Brachyura*, Section *Oxyrhyncha*) were obtained from the Supply Department at the Marine Biological Laboratory, Woods Hole. DNA was isolated from fresh tissues. Calf thymus (*Bos taurus*) was obtained fresh from a local slaughterhouse.

DNA Isolation

Our DNA isolation procedure is a composite of methods derived primarily from Marmur (1961), Smith (1963, 1964), Skinner (1967) and J. Collier (personal communication), and works reasonably well for crustacean testes and calf thymus material, from which we obtained our DNA.

Fresh tissues were placed into ice cold 0.10 M NaCl-0.20 M disodium EDTA, pH 8.0 (saline-SEDTA), and homogenized. For the crustacean materials, gentle homogenization was accomplished in several volumes of saline-SEDTA with two or three strokes in a Ten-Broeck ground glass grinder. For thymus, homogenization was accomplished by blending for 1 minute in a stainless steel Waring vessel. Concentrated sodium dodecyl sulfate was at once added to a final concentration of 2.5% (w/v), followed by addition of pronase (Calbiochem) which had been made DNase-free (Hotta and Bassel, 1965) to 100 μg/ml. The viscous solution was swirled

and incubated at 37° C for at least 1 hour, then brought to 5 M NaCl by addition of crystals. An equal volume of chloroform-isoamyl alcohol (24:1, v/v) was added, the material was shaken on a wrist action shaker for 30 minutes, and centrifuged at 10,000 rev/min for 15 minutes at room temperature. The aqueous phase was recovered with a large bore pipet and the shaking-centrifugation cycle repeated twice, by which time very little denatured protein remained standard at the interface. DNA was brought to tandard saline-citrate (1 × SSC: 0.15 M NaCl—0.015 M sodium citrate, pH 7.0) by dialysis, then treated with a mixture of α-amylase (Worthington, 25 μg/ml) and RNase (Nutritional Biochem. Corp., 50 μg/ml, made DNase-free by heating to 80° C, 30 minutes) for 30–60 minutes at 37° C, followed by DNase-free pronase (50 μg/ml) for 2 hours at 37° C. The solution was brought back to 5 M NaCl by addition of crystals, the chloroform-isoamyl alcohol shaking-centrifugation cycle was repeated twice, and the aqueous phase was recovered and dialyzed against 0.12 M phosphate buffer[1] in preparation for further purification by hydroxylapatite (HAP) chromatography. HAP (Bio-Rad) was suspended in 0.12 M PB, boiled 15 minutes, and poured into glass chromatographic columns half-filled with 0.12 M PB over 2–3 mm of washed sea sand. The bed was washed with several volumes of 0.50 M PB, returned to 0.12 M PB (buffer concentrations were continuously checked with a refractometer), and the DNA was stirred into the bed. After the DNA had run into the bed, it was washed with several volumes of 0.12 M PB, then eluted with 0.50 M PB and stored over several drops of chloroform at 4° C until used. DNA isolated in this way had a 260/230 nm ratio of 2.2 or greater; such a ratio is consistent with a protein contamination of 0.5% or less (Marmur, 1961).

Kinetics of DNA Renaturation

DNA which was to be used for kinetic analyses was brought to 0.12 M PB and sonicated on ice in 5 ml portions for 5 minutes (1 minute bursts) at full intensity with a Quigley-Rochester sonicator (titanium probe no. 12202) prior to the final HAP purification step described in the last section. The molecular weight of this double-stranded DNA was estimated by electron microscopy, using the method of Doerfler and Kleinschmidt (1970), and assuming a linear density of 1.96×10^6 daltons/μ (Thomas, 1966), which has also been confirmed by Doerfler and Kleinschmidt. The contour lengths were measured from enlarged photomicrographs projected onto a screen (Ris and Chandler, 1963), and fell into a normal distribution (Fig. 1), with a peak at 0.05–0.10 μ, corresponding to a double-stranded molecular weight of approximately 100,000–200,000 daltons. This is equivalent to a single-strand chain length range of about 165–330 nucleotides. Under similar conditions, other workers (see Saunders *et al.*, 1972) have obtained a single-strand chain length of 200 nucleotides, using alkaline sucrose gradients.

HAP-purified, highly sheared DNA was dialyzed against either 0.12 M PB or 0.50 M PB and the final DNA concentration determined by absorbance at 260 nm. A small aliquot of each DNA in 0.12 M PB was run through an HAP column operating at 60° C to determine what percent (if any) was incapable of binding at this temperature, and this value (generally 4–5%) was corrected for in subsequent calculations. This fraction possibly consists of duplexes too short to bind to heated HAP, as the absorption profile of this material is identical to DNA.

1 Throughout this paper, phosphate buffer (PB) concentrations are always prepared by mixing equal volumes of equimolar monobasic and dibasic sodium phosphate solutions. Thus, 0.12 M PB is 0.06 M monobasic sodium phosphate-0.06 M dibasic sodium phosphate, pH 6.8.

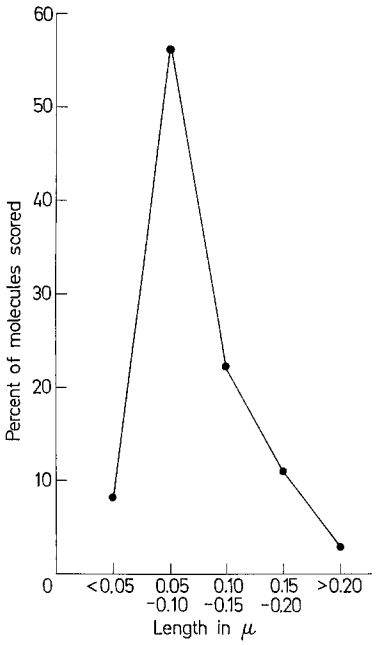

Fig. 1. Frequency distribution curve showing length distribution of sonicated double-stranded DNA molecules, as determined by measurements taken from electron micrographs

Appropriate volumes of DNA aliquots were sealed into glass capillary tubes or ampoules, denatured in a boiling water bath for 10 minutes, and incubated for appropriate times at 60° C or 62° C for DNA in 0.12 M PB and in 0.50 M PB, respectively. These incubated samples were then quickly chilled in an ice slurry and frozen until run on water-jacketed HAP columns, except for very short incubation time samples, which were run at once. Columns were prepared as described in the previous section, and 50–125 μg of DNA was stirred into the bed (0.3 g HAP: approximately 1 ml packed volume) at 60° C. It was determined that this quantity did not overload the column. Samples were always brought to 60° C before applying them to the columns, and those in 0.50 M PB were first diluted with at least 10 volumes of 0.12 M PB. Fractions of approximately 1 ml each were collected to obtain the front peak (unreassociated single-strand fraction) in 0.12 M PB and the back peak (reassociated duplex fraction) in 0.50 M PB for each incubated sample, and μg DNA/peak was calculated from the expressions: μg DNA $= A_{260} \times 40 \times$ Volume (ml) for the front peak and μg DNA $= A_{260} \times 50 \times$ Volume (ml) for the back peak. The differences in these relationships account for the difference in extinction coefficient between single-stranded and double-stranded DNA. From these values the percent DNA bound to HAP was determined for each incubated sample. The data were plotted as equivalent cot *vs* percent DNA bound to HAP, where equivalent

$$\text{cot} = \frac{\mu\text{g DNA/ml}}{83} \times \text{f} \times \text{time (hours of incubation)},$$

f being the salt correction

factor (E. Davidson, personal communication). Salt correction factors are taken from Britten *et al.* (1974). No correction was made for viscosity effects on renaturation rates at high DNA concentrations, but since we never exceeded 3.2 mg/ml and it has been shown that up to at least 3.0 mg/ml there is no detectable effect of DNA concentration on the $\text{cot}\frac{1}{2}$ value for bacterial DNA (Laird, 1971), this effect should be of little consequence, and of course our DNA samples were highly sheared.

Accurate determination of the $\text{cot}^1/_2$ value for the unique sequences is essential for calculation of kinetic complexity and genome size. This was done by solving the equation (Britten and Kohne, 1967) for an ideal second-order reaction: $\frac{C}{C_0} = \frac{1}{1+k_2\,(\text{cot})}$, where $\frac{C}{C_0}$ is the fraction of denatured DNA and k_2 is the rate constant for the reaction, for various values of cot (letting $k_2 = 1$), normalizing the resulting calculated curve to plateau at what appeared to most nearly approximate the plateau value for the slowest reassociating DNA, then laterally transposing this calculated curve to best fit the observed data points (modified from Laird and McCarthy, 1969). We have found this approach to be useful for DNAs having a well-defined unique component and this permitted accurate determination of the $\text{cot}\frac{1}{2}$ value for the unique sequences.

Results

The results of our renaturation analyses are presented in Fig. 2. The reaction for calf thymus DNA, our standard, was followed from only cot 0.1 through cot 16,000, by which time approximately 90% of the DNA had reassociated. The unique sequences comprise about 58% of the genome and have a $\text{cot}^1/_2$ of 3,900 on the average, which compares favorably with the values of 60% and $\text{cot}^1/_2$ of 4,000 obtained by Britten and Kohne (1968) using *Escherichia coli* as an internal standard. The data points show no consistent tendency to deviate from the idealized second-order curve (dashed line) above about cot 10, which we interpret to indicate that repeat frequencies of 2–350 are rare and also that no appreciable thermal degradation has occurred during the period of incubation. Incubation in 0.50 M PB permits a nearly 6-fold reduction in incubation time, and therefore would be expected to minimize thermal degradation. The plateau extends somewhat further in bovine renaturation curves published by Britten and Smith (1970), which could be due to the somewhat shorter DNA fragment lengths used in our work. Sequences reassociating between cot 10^1 to 10^{-1} comprise part of the "intermediate" rate of reassociation component. For our purposes, the most slowly reassociating (unique) portion of this curve is of greatest significance, as this is utilized to calculate kinetic complexity and genome size of the unknowns, which were treated identically. These calculations in turn depend critically on the independently determined haploid genome size of the standard; we took this to be 3.2 pg (Leuchtenberger *et al.*, 1952; Vendrely, 1955; Sandritter *et al.*, 1960).

The slowest reassociating sequences from *L. emarginata* comprise 30% of the genome, with a $\text{cot}^1/_2$ of 3,250. Since the data points show no

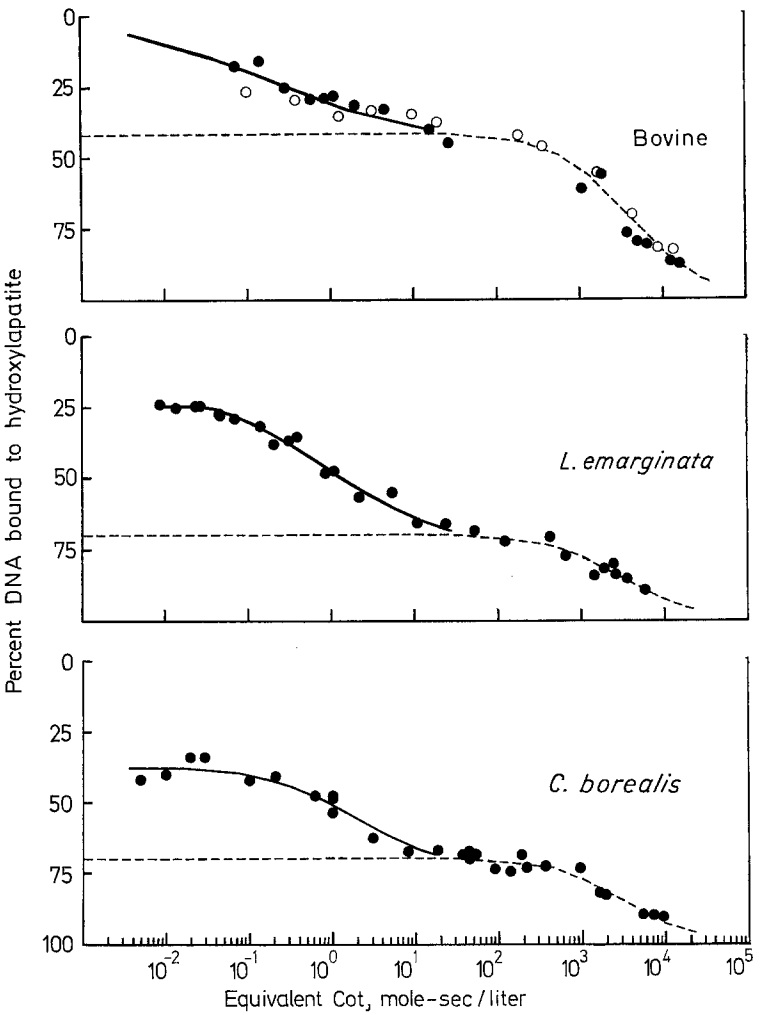

Fig. 2. Reassociation kinetics for *B. taurus* (the standard), *L. emarginata* and *C. borealis*. In each case the DNA was denatured at 100° C and allowed to renature at 60° C (for DNA in 0.12 M PB) or 62° C (for DNA in 0.50 M PB). DNA incubated at various concentrations for various times was used to attain different equivalent cot values, and aliquots in 0.12 M PB were run through hydroxylapatite columns operating at 60° C to determine the percent DNA bound, which is taken to be the percent reassociated. The dashed lines in each curve represent theoretical second-order kinetic curves which best fit the data points, as determined by a manual regression method. From top to bottom, these curves assume 58%, 30%, and 30% unique sequences, respectively. For the bovine curve the results of two independent determinations (solid circles and open circles) are plotted to show the reproducibility attained

Table 1. DNA fractions resolvable by reassociation kinetics in some higher crustaceans

Species	Percent of nuclear DNA	Multiplicity frequency range	Trivial terminology	Average multiplicity frequency
L. emarginata	30	1	slow	1
	rare	2–100	—	
	45	100–90,000	intermediate	4,000
	rare	90,000–400,000 plus	—	
	25	—	fast	
C. borealis	30	1	slow	1
	rare	2–200	—	
	32	200–70,000	intermediate	3,000
	rare	70,000–800,000 plus	—	
	38	—	fast	

consistent deviation from second-order kinetics above cot 30, we interpret this to mean that repetition frequencies from 2–100 are rare for this species. Reiteration frequencies of 100–90,000 copies are demonstrated by the next portion of the curve, comprising the "intermediately redundant" sequences. The curve levels off below cot 5×10^{-2}, and we were not able to observe reassociation kinetics much below this with our methods, although 25% of the genome had already reassociated by this cot value. This portion comprises the "fast" sequences.

The renaturation curve for *C. borealis* bears considerable qualitative similarity to that for *L. emarginata*, in that it contains a "fast" component (38% of the genome), an "intermediate" portion, and a sharply-defined slowly reassociating component (30% of the genome) whose $\text{cot}_{1/2}$ is 3,200. Reiteration frequencies of 2–200 are not detectable. These various DNA fractions are tabulated for purposes of organization and comparison in Table 1.

Discussion

Repetition Frequency Components

A survey of the literature reveals that, as a general rule, the DNA of organisms below the mammals contains an appreciable quantity of DNA with a low average reiteration frequency (often about 100 copies). This is not to say that higher levels of intragenome homology are not also present in some species. This stated generality is true for DNA from the slime mold *Dictyostelium* (Firtel and Bonner, 1972), the protozoan *Tetrahymena* (Willie, 1972), mollusks *Ilyanassa* (Davidson *et al.*, 1971) and

Crassostrea (McLean and Whiteley, 1974), arthropods *Drosophila* and *Sarcophaga* (Laird and McCarthy, 1969), *Chironomus* (Sachs and Clever, 1972), *Rhynchosciara* (Balsamo *et al.*, 1973) and *Oncopeltus* (Lagowski *et al.*, 1973), various echinoderms (Britten *et al.*, 1972; Weinblum *et al.*, 1973), and tunicates (Lambert and Laird, 1971; Weinblum *et al.*, 1973), which is not an exhaustive listing. In addition to the lower chordates cited, the generalization extends at least through the amphibians (Straus, 1971; Davidson and Hough, 1971; Flickinger *et al.*, 1973). The net effect is often a more-or-less continuous set of data points extending from unique through progressively higher and higher reiteration families. The various "discrete DNA classes" must often be separated by computer analysis, so that it may be difficult to obtain a clean "single-copy" fraction[2]. This is in sharp contrast to the situation in mammals, in which reiterations of 2 to several hundred (even as much as one thousand) copies are often rare. For example, this is (in our opinion, based on examination of published data only) apparently the case for DNA from the common laboratory mouse (McConaughy and McCarthy, 1970; Laird, 1971), rat (Holmes and Bonner, 1974), rabbit (Schultz *et al.*, 1973), cow (Britten and Kohne, 1968), and human (Saunders *et al.*, 1972), although human may contain a few hundred copy repeated elements (Britten, personal communication). We have shown in the present communication that the crabs *L. emarginata* and *C. borealis* contain few detectable repeated sequences in the frequency range 2–100 copies, but many copies in the low hundreds range. This is unusual but not unknown for invertebrate DNAs: for example examination of published renaturation curves for tunicate DNA (Lambert and Laird, 1971) reveals that these appear to contain few repeated sequences in the approximate range 2–80. Absence of low reiteration families in the two crab species suggests that if new sequences do arise by "saltatory replications" (Britten and Kohne, 1968), then they are not often added here in numbers less than about 100 copies. The presence of intermediate component multiplicity frequencies up to 90,000 copies in crabs is unusual for invertebrates, but not unknown. The moth *Bombyx* contains a component with an *average* of 50,000 copies (Gage, 1974), and of course satellites are present in many species at much higher multiplicity frequencies.

In attempting to account for the lower multiplicity frequency of *Drosophila vs* mammalian DNAs, Laird and McCarthy (1969) postulated that the relatively simple genome of dipteran insects may be correlated

2 This compounds a problem which is always present in such studies anyway, namely the arbitrary criteria used to distinguish between "single-copy" and repetitious DNA sequences. This distinction is recognized to be in part artificial, and depends upon the reaction conditions which are chosen (Britten and Davidson, 1971; McCarthy and Farquhar, 1971).

with higher mutation rates so that "if the rate of divergence by fixation of mutations exceeds the rate of creation of new intragenome homologies by gene duplication, apparent genome simplicity would result." This correlates with the rapid loss of DNA base sequence relatedness for *Drosophila* species which has in fact been observed (Laird and McCarthy, 1968; Entingh, 1970; Laird, 1973). Conversely, if the rate of divergence by fixation of mutations did not exceed the rate of creation of new intragenome homologies, this could account for the observed conservation of sequence relatedness in mammalian DNAs (Hoyer *et al.*, 1964, 1965). To whatever extent these considerations are valid, one could expect to find a similar correlation of the lack of very low multiplicity frequencies in crustacean DNAs with the considerable redundant component base sequence conservation which has been reported (Graham and Skinner, 1973; Vaughn, 1973; Vaughn and Bachmann, 1973; Vaughn and Traeger, 1975). However, given differences and the uncertainties in generation times (Kohne *et al.*, 1971), relative rates of accumulation of base substitutions (Laird *et al.*, 1969), and addition of new sequences to genomes (Rice, 1971a, b), not to mention the very small number of different groups of organisms so far examined, it is difficult if not impossible to evaluate this interesting question at this time. Another explanation for the discontinuous distribution of sequence frequencies could involve gene regulation, which has been advanced as an important hypothesis by Britten and Davidson (1969).

We should also comment on the large proportion of "fast" DNA sequences observed for the two crab species. Thermal elution of sheared *L. emarginata* and *C. borealis* DNA from a hydroxylapatite column yields a dAT satellite fraction of approximately 8.0 and 27.0%, respectively (Vaughn and Bieber, unpublished results). Sueoka (1961) and Cheng and Sueoka (1964) originally reported that *C. borealis* contains about 30% dAT satellite in CsCl gradients. This component would be expected to renature very rapidly, as its base sequence is largely alternating deoxyadenylate and deoxythymidylate (Swartz *et al.*, 1962). Therefore, a portion of the "fast" sequences reported in the present communication would represent the dAT satellite resolvable by CsCl density centrifugation or by thermal chromatography. Satellites which are (G + C)-rich have also been reported to be present in these and related species in small amounts: 3% for *L. emarginata* (Vaughn and Hinsch, 1970); 2% for *L. dubia* and none detected for *C. borealis* (Skinner *et al.*, 1970). It is striking that the proportion of the genome of each crab found to be "fast" in the present study is nearly identical to the proportion found by thermal denaturation in spectrophotometer cuvettes to be early-denaturing and therefore (A + T)-rich, despite the fact that a much smaller (A + T)-rich satellite is resolvable in CsCl. This could be due to either: (1)

the presence of some very short satellite sequences scattered widely throughout the genome and thus unresolvable in CsCl gradients, or (2) the presence of mutated satellite components, or (3) the presence of sequences related to the dAT satellite in some other way (Vaughn, 1972). Our data on early-denaturing (A + T)-rich sequences in *Crustacea* will be reported elsewhere.

Chromatid Structure and Genome Size

We were especially interested in determining whether there exists a strong correlation between kinetic complexity and independently derived haploid genome size, as this correlation has been utilized to demonstrate uninemic chromatid structure in some organisms (Laird, 1971). In Tables 2 and 3 the essential values are tabulated which permit an assessment of this question. The percent G + C values of the respective DNAs were obtained from Sober (1970) for *B. taurus* (42%), and from recalculation of Cheng and Sueoka's data (1964) for *C. borealis* (37%). The main band component of *C. borealis* and another *Libinia* species (*L. dubia*) have been shown to have the same percent base composition as revealed by their density in neutral CsCl gradients (Skinner *et al.*, 1970) and we have assumed that *L. dubia* and *L. emarginata* would be rather similar in percent G + C content. The haploid DNA value of 2.2 pg for *L. emarginata* was taken from Rheinsmith *et al.* (1974) and Table 2; the value of 2.15 pg for *C. borealis* was determined by Bachmann and Vaughn (unpublished results) and Table 2. Feulgen microspectrophotometry was utilized in all cases. Calculation of haploid genome size for *L. emarginata* from kinetic data reveals that the ratio: corrected bovine $\text{cot}\frac{1}{2}$/corrected *L. emarginata* $\text{cot}\frac{1}{2} = 1.39$. Therefore the bovine genome, whose chromatids are thought to be uninemic (Laird, 1971), should be 1.39 times as large as this crab genome *if* the crab chromatids are uninemic, so that: 1.92×10^{12} daltons = (1.39) (x). Solving, the haploid *Libinia* genome size is calculated to be 1.38×10^{12} daltons, which corresponds to 2.30 pg of DNA. This is in fact precisely the haploid value obtained by photometry (Table 2). Therefore this haploid genome contains insufficient DNA to support a binemic or polynemic chromatid structure, or its equivalent. A similar calculation for *C. borealis* yields 1.36×10^{12} daltons, corresponding to 2.27 pg. The nearly exact correlation between haploid genome size and kinetic complexity leads to the conclusion that the sequences present in the most slowly renaturing (*i.e.* unique) component are present only once per haploid genome in both species, and therefore that the chromatids of these species are uninemic, at least with respect to the unique portion of the genome. Uninemic chromatid structure in another arthro-

Table 2. Microspectrophotometric determination of nuclear DNA content in different diploid blood cells[a]

Species	Number measured	Mean DNA ($\times 10^{-12}$ g)	
		Experimental value	Literature value
Rana pipiens (erythrocytes)	31	—[b]	15.0 (Bachmann, 1970)
Callinectes sapidus (hemocytes)	51	—[b]	4.2 (Rheinsmith *et al.*, 1974)
Cancer borealis (hemocytes)	34	4.0–4.3[c]	—
Libinia emarginata (hemocytes)	37	4.4–4.6[c]	4.4 (Rheinsmith *et al.*, 1974)

[a] All determinations made on formaldehyde-fixed, Feulgen-stained smears precisely as described in detail by Vaughn and Locy (1969).
[b] Used literature values as standards of comparison.
[c] The ranges reflect the extent of the standard errors of the experimentals when compared to the standards, which are taken at face value.

Table 3. Comparison between kinetic complexity and haploid DNA content per cell

Species (% G + C)[a]	% unique	Cot $^1/_2$ (mole-sec/liter)		Haploid genome, daltons (pg)	
		Corrected for repeated sequences[b]	Corrected to 50% G + C[c]	Calculated from kinetics[d]	Literature value[a]
B. taurus (42)	58	3,900 (4)	3,120	—	1.92×10^{12} (3.2)
L. emarginata (37)	30	3,250 (3)	2,243	1.38×10^{12} (2.30)	1.32×10^{12} (2.2)
C. borealis (37)	30	3,200 (2)	2,208	1.36×10^{12} (2.27)	1.29×10^{12} (2.15)

[a] Literature values for % G + C are given in the text; for genome sizes see Table 2 and text.
[b] The number of independent determinations is given in parentheses. Owing to the small number of samples, no attempt has been made to treat these values statistically.
[c] Determined according to Wetmur and Davidson (1968).
[d] See text.

pod (various species of *Drosophila*) has also been recently proposed based on studies of viscoelastic relaxation of the largest DNA molecules recoverable from chromosomes (Kavenoff *et al.*, 1973).

We would expect that, had these crab species arisen by some sort of polyploidy mechanism, as has been postulated for certain other crustacean species (Bachmann and Rheinsmith, 1973), then sequences from even the "unique" portion of the renaturation curves should have contained two (or more) similar or identical copies of DNA per genome and the corrected $\text{cot}\frac{1}{2}$ values for these crabs would have been approximately 1,100 (or less), which was clearly not the case. This should however be a fruitful approach for evaluating the evolution by polyploidy hypothesis if appropriate species are selected for analysis. For reviews of recent approaches utilized for the determination of chromosome structure by this and alternative methods, see Ris and Kubai (1970), Prescott (1970), and Laird (1973).

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